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Official Organ of the

American Phytopathological Society

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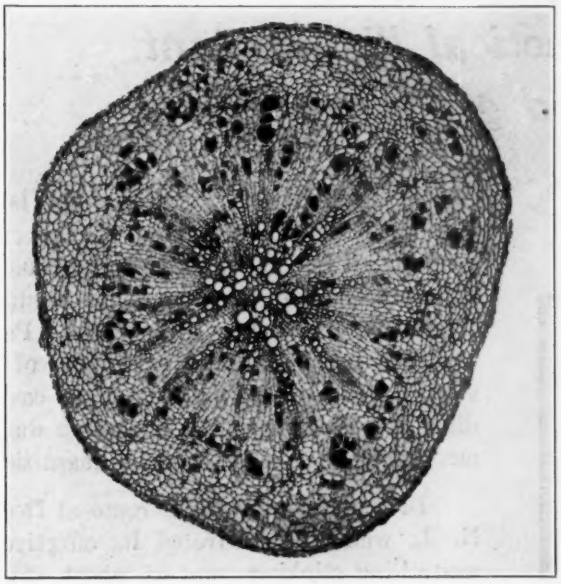

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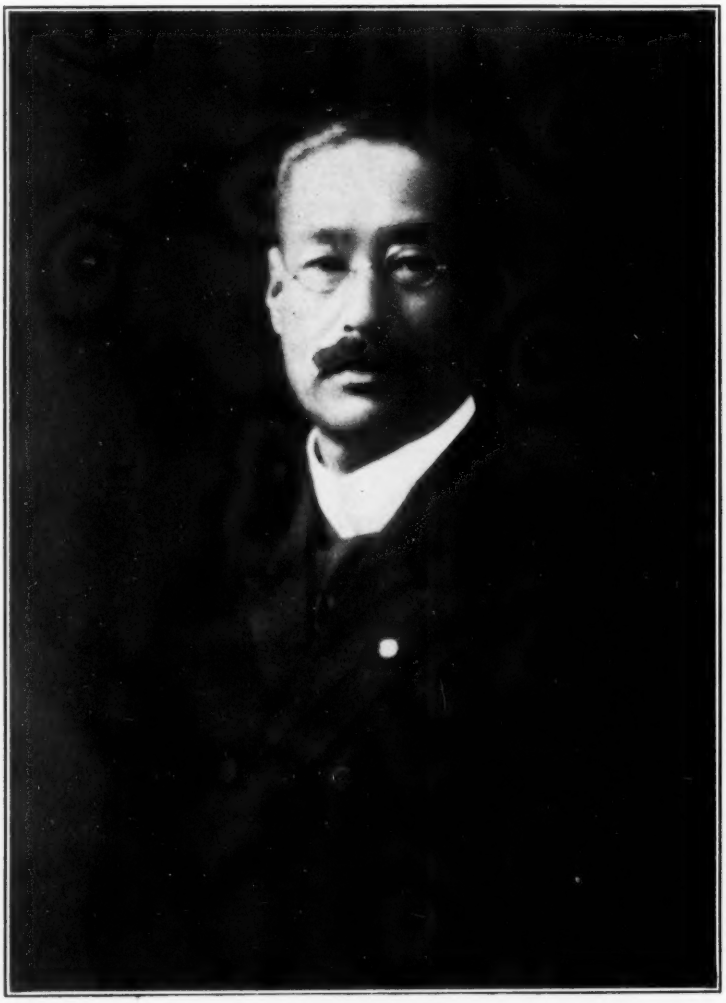

TŌJI NISHIDA

PHYTOPATHOLOGY

VOLUME 19 NUMBER 10

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TŌJI NISHIDA
1874–1927

R. KENT BEATTIE[1]

Dr. Tōji Nishida was born in Tokyo, Japan, on April 15, 1874. He died at Kumamoto on June 19, 1927. He belonged to a distinguished family, his father having been a Court poet and his father-in-law a jurist and member of the Diet.

Dr. Nishida completed the course at the Sapporo Agricultural College (now the Hokkaido Imperial University) in 1899, from which institution he received the degree of Bachelor of Agriculture. He was a pupil of Dr. Kingo Miyabe, known to so many plant pathologists in the United States. His baccalaureate thesis was entitled: "Studies on the Japanese species of Exoascaceae" and his interest in this fungus group was retained throughout life. In 1919 he became a Doctor of Agriculture.

After graduation from the Sapporo Agricultural College, he was at once appointed a member of the staff of the Imperial Agricultural Experiment Station at Nishigahara near Tokyo where he served till 1904 when he was transferred to the Kyushu branch of the Imperial Experiment Station located at Kumamoto. During the Russo-Japanese war he served with the Japanese Army, returning to the Kumamoto Station in 1906.

In 1914 he was promoted to Plant Pathologist and became the Director of the Kobe Substation of the Imperial Plant Quarantine Service. In 1923 he left the Quarantine Service and returned to the Kyushu Branch Experiment Station. With the closing of this station in 1924, Dr. Nishida entered the Kumamoto Medical College as a student with the purpose of acquiring a sufficient knowledge of human diseases to permit him to enter the field of Comparative Plant and Animal Pathology. However, before his course was completed, apoplexy laid him low at the age of fifty-three. He is survived by his wife and two sons.

Dr. Nishida was interested chiefly in cereal and fruit diseases, more especially in citrus diseases and the Exoascaceae. He was the author of at least forty-two papers the titles of which are listed in the appended bibliography.

[1] The data herein have been kindly furnished by Dr. Nishida's eldest son, Mr. Masaaki Nishida.

He was author or original collector of the following species of fungi: *Taphrina alni-incanae* Nishida, *T. alni-japonicae* Nishida, *T. betulicola* Nishida, *T. coryli* Nishida, *T. hiratsukae* Nishida, *T. mume* Nishida, *T. struthiopteris* Nishida, *Gloeosporium foliicolum* Nishida, *Triphragmium nishidanum* Diet., *Puccinia nishidana* P. Henn.

Dr. Nishida's worth to Japan was recognized by the conferring upon him of the "Second Grand Decoration of the fourth rank, and the fourth Order of Merit."

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PATHOGENICITY AND CULTURAL BEHAVIOR OF *USTILAGO ZEAE* (BEKM.) UNG. FROM DIFFERENT LOCALITIES[1]

A. H. EDDINS[2]

INTRODUCTION

In developing high yielding synthetic varieties of corn, it is essential that inbred strains making up such varieties be free of disease if maximum yields are to be obtained. The recent work of Christensen and Stakman (3) on *Ustilago zeae* (Bekm.) Ung. indicates that the corn breeder is now confronted with the question as to whether or not a particular synthetic variety of corn, resistant to the smut of the section where it is developed, will be resistant to that of other sections where this variety may be grown.

The studies reported in this paper were undertaken to determine whether *Ustilago zeae*, as it occurred throughout Iowa, differed in its ability to produce smut on plants of selected inbred lines of corn. This has involved mainly a detailed study of the pathogenicity and cultural behavior of *U. zeae* collected from twelve different localities in the State.

REVIEW OF LITERATURE

Physiologic specialization has been observed in several genera of the smut fungi (11, 31, 32, 33, 42). Melchers (26) indicated the presence of physiologic differences in *U. zeae*. He found that selections and crosses of corn resistant in one locality or State were much more susceptible in other sections. Tisdale and Johnston (40) also found that cultures of *U. zeae* from some sources were much more virulent than cultures of the organism from other sources. Stakman and Christensen (38) obtained evidence of physiologic forms of *U. zeae*, and later these authors (3) came to the conclusion that *U. zeae* is a group species made up of numerous physiologic forms. Finally, Griffiths (14) reported that six different collections of corn smut from the Arlington Experiment Farm showed differences in culture and pathogenicity.

Recent investigations point to the fact that certain fungi behave as if sexual strains were present. Blakeslee (1) found both heterothallism and homothallism in the Mucors. Dodge (9) produced fertile hybrid perithecia in culture by crossing two species of the red bread mold fungi, *Neurospora*

[1] This paper is the major part of a thesis submitted to the graduate faculty of Iowa State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy, granted December 21, 1928.

[2] Grateful acknowledgment is made to Drs. J. B. Wentz, E. W. Lindstrom, and J. C. Gilman for criticisms and suggestions; and to Mr. D. G. A. Kelbert for making all photographs.

sitophila (Mont.) Shear and B. O. Dodge and *N. tetrasperma* Shear and B. O. Dodge. Craigie (4, 5), while working with *Puccinia helianthi* Schw. and *P. graminis* Pers., found that sex existed in the rust fungi. Different workers (17, 23, 28, 43) have reported the quality of sex in several species of the Hymenomycetes.

Tisdale, Melchers, and Clemmer (41) advanced the hypothesis that a new form of smut in milo and hegaria sorghums arose through the hybridization of *Sphacelotheca sorghi* (Lk.) Clint. and *S. cruenta* (Kuehn.) Potter by fusion between hyphae of the two species growing in the same host plant. Kniep (22) reported conjugation occurring between germ tubes of different species of Ustilago. Dickinson (6, 7) reported sexual strains in *Ustilago levis* (Kell. and Sw.) Magn. and *U. hordei* (Pers.) Kell. and Sw. He discovered that two of the four isolated sporidia of a single chlamydospore were of one sex and two of another sex. Stakman (37) concluded that *U. zeae* was heterothallic, as the fusion of two multisporidial strains of opposite sex was necessary for the production of chlamydospores in the host plant.

Workers differ in their opinions as to where sexual fusions occur in *U. zeae*. Maire (25), Hitchcock and Norton (16), and Sartoris (34) have observed fused sporidia. Lutman (24) was unable to determine how the multinucleated vegetative mycelium developed into the chlamydospore. Rawitscher (30), Seyfert (36), and Stakman (37) have observed what they thought to be sexual fusions in *U. zeae* growing in the host plant prior to the formation of chlamydospores.

MATERIALS AND METHODS

Materials. Inbred lines of corn were used in these studies, for they differ from each other in smut susceptibility (12, 15, 19, 20, 21). The lines[3] had been grown from three to five years in the experimental plots at Ames and notes on smut infection had been taken on most of them each year. The inbreds, with their parents and smut reaction, are shown in table 1.

The twelve collections of smut used in this work obtained from the sources indicated in table 2 and the accompanying map, figure 1. In addition to these collections, five cultures isolated from one smut gall were used and designated A1, A2, A3, A4, and A5. Emery R. Ranker, of the U. S. Dept. of Agr., Washington, D. C., furnished six monosporidial cultures known as Wisconsin 1, Arlington Farm 2, Michigan 10, Arlington Farm 14, South Dakota 37, and Kansas 38.

Methods. Pure cultures of *U. zeae* were obtained in two ways: (1) Chlamydospores were soaked in a 1 per cent solution of copper sulphate

[3] These were obtained from E. W. Lindstrom and Merl T. Jenkins, of the Iowa Agricultural Experiment Station, Ames, Iowa.

TABLE 1.—*Data showing smut reaction of inbred lines of corn used in the smut infection studies*

Variety	Inbred line number	Number years selfed	Percentage smut				
			1923	1924	1925	1926	1927
Four County White	5	5	—[a]	10	60	10	30
Four County White	35	5	—	20	40	30	20
Four County White	97	5	0	0	0	0	20
C. I. No. 133	134	5	10	30	50	10	65
Iodent	195	4	0	0	0	0	7
Iodent	224	5	—	20	30	10	50
Iodent	224[a]	5	—	20	60	10	30
Iodent	238	5	0	0	0	0	0
C. I. No. 204	273	5	—	10	40	—	50
Lancaster Sure Crop	311	4	0	0	0	—	0
Black's Yellow Dent	344	5	0	0	0	0	55
Black's Yellow Dent	356	5	—	20	40	20	64
Reid's Yellow Dent, Proudfit's strain	358	5	—	10	50	20	50
Krizer Bros. Yellow Dent	379	5	—	20	50	0	30
Osterland Yellow Dent	420	4	0	0	0	—	21
Clark's Yellow Dent	440	5	0	0	0	0	0
Clark's Yellow Dent	440[a]	5	0	0	0	0	7
Walden's Yellow Dent	452	5	10	30	60	25	50
Walden's Yellow Dent	460	5	0	0	0	0	0
Evergreen Sweet	S314	3	0	0	0	0	5
Golden Bantam Sweet	6883	3	0	0	0	0	10

[a] No notes taken on smut infection.

TABLE 2.—*Sources of twelve collections of Ustilago zeae in Iowa*

Collection	Locality	Collector
1	George, Lyon Co.	James C. Halasey
2	Buffalo Center, Winnebago Co.	Fred G. Bliss
3	Postville, Allamakee Co.	Fred C. O'Riley
4	Cherokee, Cherokee Co.	Lester M. Blanch
5	Goldfield, Wright Co.	Miller S. Nelson
6	Manchester, Delaware Co.	Earl New
7	Castana, Monona Co.	Carl D. Kiser
8	Ames, Story Co.	A. H. Eddins
9	Center Junction, Jones Co.	Paul N. Smith
10	Elliott, Montgomery Co.	J. H. Petty
11	Lamoni, Decatur Co.	F. S. Parks
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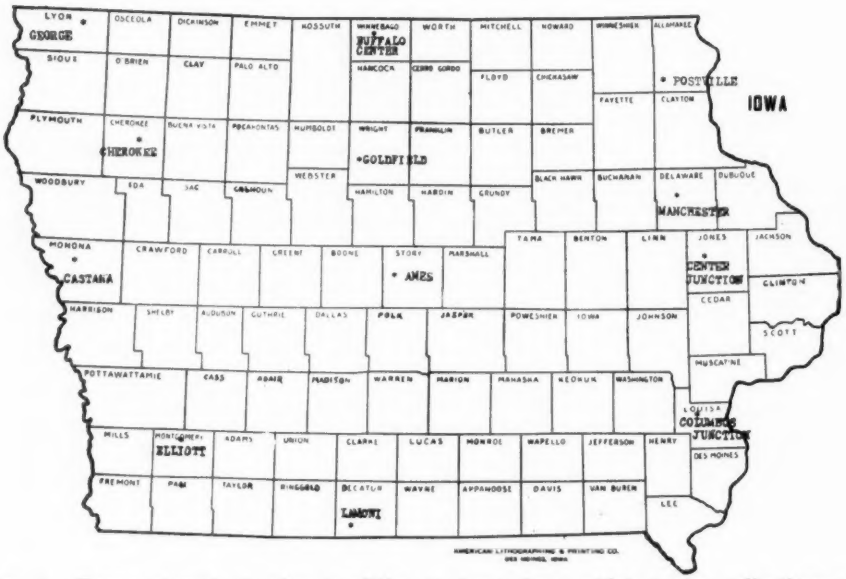

FIG. 1. Towns starred showing localities in Iowa from which twelve collections of *Ustilago zeae* were made.

from 12 to 60 hours, and a pure culture was developed by removing a little of the spore suspension with a sterile platinum loop and streaking it across a carrot agar slant in a test-tube (3). (2) Pure cultures of the fungus were

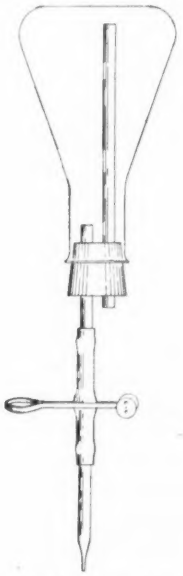

FIG. 2. Erlenmeyer flask used in dropping inoculum on apical buds.

isolated from the interior of fresh unbroken smut galls. The fungus was grown on a 3 per cent carrot agar, and conidial suspensions for inoculation were prepared by growing the conidia in carrot decoction in Erlenmeyer flasks from six to ten days.

The plants were inoculated by two methods: (1) Conidial suspensions of *U. zeae* were injected into young plants with a hypodermic syringe when the plants were in the three- to four-leaf stage (40). (2) Conidial suspensions were dropped on the apical buds of plants which averaged a foot in height (2, 14, 16, 40). A pipette was used to drop inoculum into the tops of plants grown in the greenhouse, and an Erlenmeyer flask fitted with clamp, rubber, and glass tubes, as is shown in figure 2, was used in field inoculations.

GREENHOUSE EXPERIMENTS

Inoculation experiments were conducted in the greenhouse in the winter of 1927–28, at Ames, Iowa. Inbred lines which had proven to be susceptible, moderately susceptible, and resistant to smut under natural field conditions at Ames were used in these experiments. The corn was grown in greenhouse flats and in flower pots. Plants grown in flats were inoculated hypodermically, and those in pots by dropping the inoculum on the apical buds. The greenhouse, kept moist by a thorough spraying twice daily, was maintained at a mean temperature of 78° F.

Pathogenicity of cultures of U. zeae from twelve different localities in Iowa. The results obtained from inoculating ten inbred lines of corn by the hypodermic and dropper methods are given in tables 3 and 4, respectively. The percentage of plants infected includes those with galls and those that developed chlorotic and necrotic areas on the leaves, leaf sheaths, and stalks of the young seedlings. The data, presented in these two tables and graphically shown in figure 3, show that the twelve cultures differed from each other in the percentage of plants infected and the percentage of plants with galls in each of the ten inbred lines of inoculated corn. The check plants were inoculated with sterile carrot decoction and none of them developed smut galls or became chlorotic or necrotic.

A summary of the results of the inoculations is given in table 5. When the ten inbreds were inoculated hypodermically, cultures 3, 6, 8, 9, and 12 each produced galls on from seven to nine lines of corn. Cultures 1, 2, 4, 10, and 11 are in a group which formed galls on from two to four inbreds out of the total of ten inoculated. Culture 5 produced galls on one line of corn and culture 7 failed to form galls by the hypodermic method of inoculation.

When the inoculum was dropped on the apical buds of plants, the results differed from those obtained when the same inbreds were inoculated hypodermically with the same smut cultures. The data in table 5 show that cultures 8 and 6 formed galls on seven and eight lines, respectively. Galls were

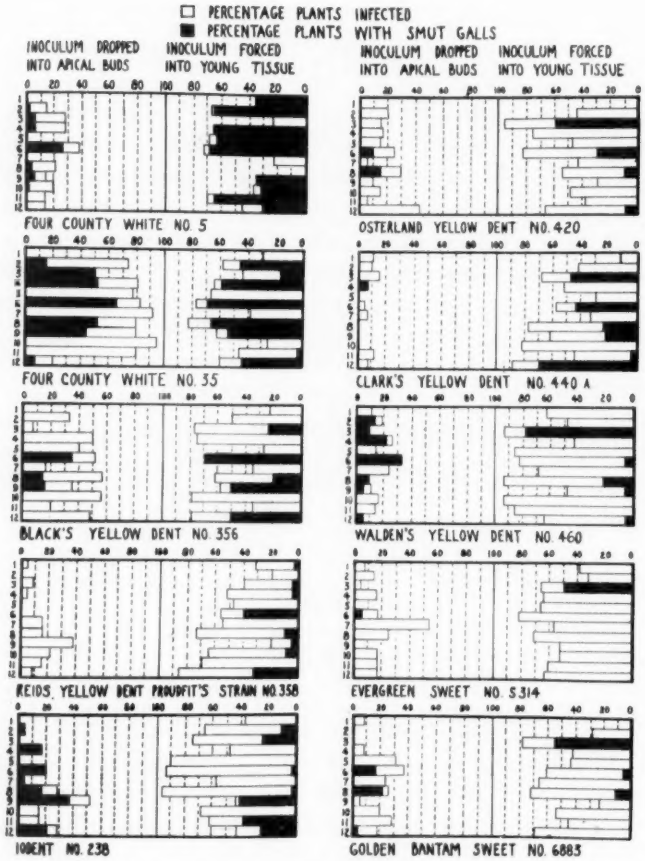
PERCENTAGE PLANTS INFECTED
PERCENTAGE PLANTS WITH SMUT GALLS
INOCULUM DROPPED INTO APICAL BUDS
INOCULUM FORCED INTO YOUNG TISSUE
INOCULUM DROPPED INTO APICAL BUDS
INOCULUM FORCED INTO YOUNG TISSUE
FOUR COUNTY WHITE NO. 5
OSTERLAND YELLOW DENT NO. 420
FOUR COUNTY WHITE NO. 35
CLARK'S YELLOW DENT NO. 440 A
BLACK'S YELLOW DENT NO. 356
WALDEN'S YELLOW DENT NO. 460
REIDS YELLOW DENT PROUDFIT'S STRAIN NO. 358
EVERGREEN SWEET NO. S 314
IODENT NO. 238
GOLDEN BANTAM SWEET NO. 6883

FIG. 3. Results of inoculating ten inbred lines of corn with twelve cultures of *Ustilago zeae*. Cultures of *U. zeae* Nos. 1 to 12 appear on left and percentage smut at the top of each histograph.

produced on three to five inbreds when inoculated with cultures 2, 3, 4, 9, and 12. Cultures 7 and 10 each produced galls on only one line and no galls were formed when cultures 1, 5, and 11 were used.

The inbred lines were used as differential hosts (3, 40) and the results show that there is a difference in the pathogenicity of the cultures of *U. zeae* from different localities in Iowa, for several cultures produced galls on only two or three lines or not at all, while other cultures formed galls on from seven to nine inbreds.

Pathogenicity of five cultures of U. zeae isolated from one smut gall. The results of inoculating six inbreds with five cultures of *U. zeae* from the same smut gall are reported in tables 6 and 7 and graphically shown in

TABLE 3.—*Results of inoculating ten inbred lines of corn with twelve cultures of Ustilago zeae by forcing the inoculum into the plants with a hypodermic syringe*

	Four County White No. 5			Four County White No. 35			Black's Yellow Dent No. 356			Reid's Yellow Dent Proudfit's Strain No. 358			Iodent No. 238			Osterland Yellow Dent No. 420			Clark's Yellow Dent No. 440a			Walden's Yellow Dent No. 460			Evergreen Sweet No. S314			Golden Bantam Sweet No. 6883		
Smut culture	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls
1	31	36	36	24	29	0	13	23	0	29	31	4	25	36	0	25	0	0	28	12	0	27	63	0	25	39	0	16	0	0
2	34	67	65	26	58	46	20	50	0	24	20	0	25	68	12	16	44	0	27	41	0	27	48	0	25	32	0	21	29	0
3	35	23	0	25	44	16	25	77	24	22	40	5	20	75	25	22	95	59	28	68	47	28	93	78	24	66	50	18	78	55
4	33	66	66	22	64	59	16	75	0	21	52	5	23	48	0	16	75	0	27	52	0	28	43	0	22	64	0	21	43	0
5	35	69	63	24	66	0	26	27	0	19	47	0	16	90	0	17	47	0	27	29	0	28	85	0	27	66	0	18	44	0
6	32	72	69	13	77	69	26	69	69	23	56	40	27	93	4	17	82	29	28	57	43	28	82	7	22	82	0	15	60	6
7	37	22	0	21	38	0	26	35	0	22	55	0	21	57	0	24	42	0	28	32	0	27	69	0	28	57	0	18	66	0
8	35	0	0	12	83	66	24	62	20	19	74	10	23	96	4	22	54	9	26	77	23	27	93	22	24	71	0	18	72	11
9	31	35	35	24	62	54	26	58	50	20	20	5	23	43	40	17	29	0	28	61	21	27	48	7	27	52	0	22	23	0
10	35	37	31	23	26	0	24	79	0	23	65	9	22	68	0	23	48	0	26	81	0	27	93	0	27	52	0	24	54	0
11	37	70	65	24	46	4	23	35	0	28	69	0	21	62	38	19	37	0	27	44	4	28	85	0	25	60	0	22	45	0
12	40	45	30	25	60	44	24	79	50	22	86	32	24	42	25	21	66	9	26	88	69	28	64	7	24	62	0	26	69	0
Check	40	0	0	18	0	0	26	0	0	19	0	0	23	0	0	18	0	0	26	0	0	28	0	0	27	0	0	19	0	0

TABLE 4.—*Results of inoculating ten inbred lines of corn with twelve cultures of Ustilago zeae by dropping the inoculum on the apical buds of the plants*

Smut culture	Four County White No. 5			Four County White No. 35			Black's Yellow Dent No. 356			Reid's Yellow Dent Proudfit's Strain No. 358			Iodent No. 238			Osterland Yellow Dent No. 420			Clark's Yellow Dent No. 440a			Walden's Yellow Dent No. 460			Evergreen Sweet No. S314			Golden Bantam Sweet No. 6883		
	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls
1	47	0	0	26	0	0	14	0	0	23	4	0	17	0	0	12	0	0	25	11	0	18	11	0	27	7	0	27	8	0
2	50	14	2	23	74	15	21	33	0	22	0	0	23	4	4	20	20	0	28	10	0	21	19	14	28	14	0	24	0	0
3	48	28	6	22	50	50	13	7	0	23	9	0	21	0	0	20	15	0	27	15	0	20	10	10	23	4	0	27	0	0
4	46	28	6	27	81	52	24	50	0	21	4	0	23	17	17	18	17	0	27	7	7	23	26	22	25	16	0	25	8	0
5	49	0	0	26	77	0	21	40	0	25	0	0	23	0	0	20	15	0	28	0	0	21	14	0	22	0	0	26	31	0
6	50	38	26	24	83	66	23	52	35	19	0	0	25	20	20	20	25	10	27	4	0	24	33	33	24	17	5	24	37	17
7	49	20	0	25	92	0	18	16	0	24	16	0	25	4	4	19	5	0	28	7	0	21	24	0	26	54	0	21	24	0
8	48	21	4	25	80	52	21	57	14	18	16	0	24	29	17	20	30	15	28	0	0	22	9	9	24	25	0	23	26	22
9	50	14	6	25	80	44	20	35	15	26	38	0	21	52	38	20	10	0	28	0	0	19	11	5	26	0	0	22	5	0
10	46	19	2	22	95	0	16	56	0	27	22	0	20	15	0	20	15	0	26	0	0	19	16	0	23	17	0	25	20	0
11	45	13	0	25	80	0	25	20	0	26	15	0	20	15	0	21	5	0	25	12	0	21	14	0	28	17	0	24	29	0
12	46	13	0	15	20	7	21	48	0	22	9	0	24	29	21	21	43	0	25	8	0	20	5	5	24	17	0	26	19	4
Check	46	0	0	27	0	0	23	0	0	25	0	0	22	0	0	15	0	0	24	0	0	21	0	0	25	0	0	26	0	0

TABLE 5.—*Number of lines of corn showing necrosis, chlorosis, and galls when inoculated with twelve smut cultures hypodermically and by dropping the inoculum on the apical buds*

Method of inoculation	Number of lines inoculated	Number lines infected when inoculated with											
		No. 1		No. 2		No. 3		No. 4		No. 5		No. 6	
		Nec.[a] Clo.	Galls present	Nec. Clo.	Galls present	Nec. Clo.	Galls present	Nec. Clo.	Galls present	Nec. Clo.	Galls present	Nec. Clo.	Galls present
Inoculum forced into young tissue hypodermically	10	8	2	10	3	10	9	10	3	10	1	10	9
Inoculum dropped into apical buds	10	5	0	8	4	8	3	10	5	5	0	9	8

Method of inoculation	Number of lines inoculated	Number lines infected when inoculated with											
		No. 7		No. 8		No. 9		No. 10		No. 11		No. 12	
		Nec. Clo.	Galls present	Nec. Clo.	Galls present	Nec. Clo.	Galls present	Nec. Clo.	Galls present	Nec. Clo.	Galls present	Nec. Clo.	Galls present
Inoculum forced into young tissue hypodermically	10	10	0	9	8	10	7	10	2	10	4	10	8
Inoculum dropped into apical buds	10	10	1	9	7	8	5	9	1	10	0	10	4

[a] Nec., necrotic; Clo., chlorotic.

figure 4. In a few cases, a culture of smut produced galls on plants of an inbred when inoculated by one method, but failed to form galls on the same inbred when inoculated by the other. Plants infected with smut when inoculated by the dropper method are shown in figure 5. The five cultures from the same smut gall differed from each other in the percentage of plants infected and the percentage with galls when the six inbreds were inoculated.

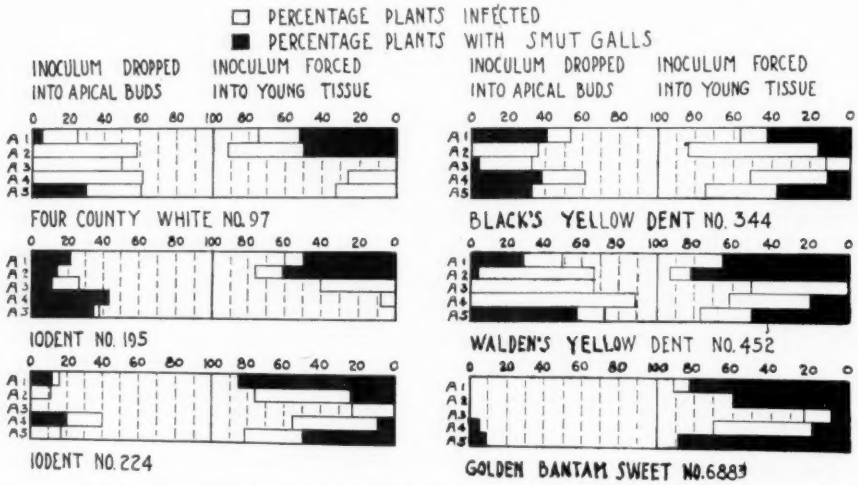

FIG. 4. Results of inoculating six inbred lines of corn with five cultures of *U. zeae* from the same smut gall. Cultures A1 to A5 appear on left and percentage smut at top of each histograph.

The data in table 8 are a summary of the results of the inoculations. When the seedlings were inoculated hypodermically, cultures A1 and A2 produced galls on all six lines. Cultures A4 and A5 each formed galls on four inbreds and A3 produced galls on only one line. When the inoculum was dropped into the tops of the plants, A1 and A5 each produced galls on five lines. A4 formed galls on four lines and A2 and A3 each produced galls on only two inbreds. The cultures from the same smut gall differed from each other in their ability to produce smut about as much as did the twelve cultures isolated from galls collected in different localities in Iowa. The results not only indicate that *U. zeae* in the same locality (3, 14) varies in its pathogenicity, but they also show that a single smut gall may be composed of a great number of pathogenic variants of the organism, as indicated by Griffiths (14).

Pathogenicity of monosporidial cultures and their combinations. Six single-sporidial cultures of *U. zeae* were obtained from Emery R. Ranker, Washington, D. C. Seedlings of five inbred lines of corn were inoculated hypodermically with these monosporidial cultures and their paired combina-

tions. The combinations were made at the time of inoculation by mixing equal quantities of the inoculum of each monosporidial culture of which the combination consisted. The results of the inoculations are given in table 9. One monosporidial culture, South Dakota 37, produced chlamydospores in host tissue, and the other five cultures did not. The results of this pathogenicity test led to the question as to whether *U. zeae* was heterothallic or homothallic or both. The production of chlamydospores in corn plants inoculated with paired monosporidial cultures and the absence of chlamydospores in plants inoculated with such cultures alone served as the criterion for

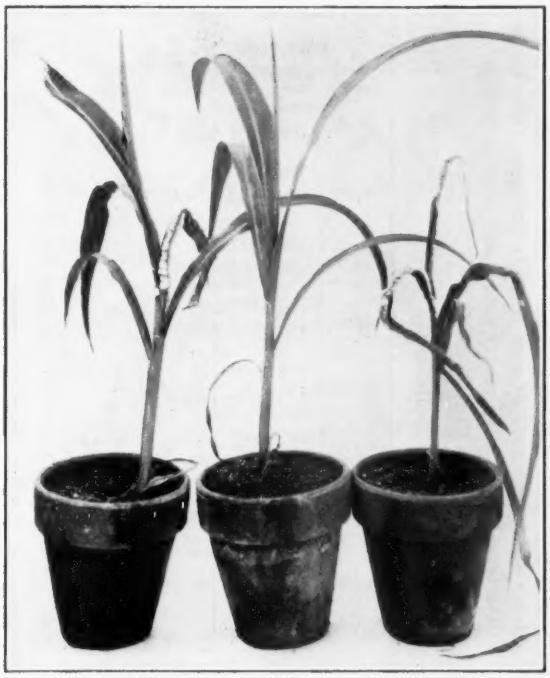

FIG. 5. Two outer plants infected with smut and center plant not infected when inoculated by the dropper method in the greenhouse.

heterothallism (37). A monosporidial culture was considered homothallic if it alone produced chlamydospores in the host plant.

Previous workers (17, 28) have found that cultures of certain Hymenomycetes, which are regularly heterothallic, changed spontaneously to the homothallic condition. The fact that only one of the six monosporidial cultures of *U. zeae* produced chlamydospores in plants inoculated with the cultures alone suggests that this organism also is regularly heterothallic and that those monosporidial cultures which produce chlamydospores in host

TABLE 6.—*Results of inoculating six inbred lines of corn with five cultures from the same smut gall by injecting the inoculum into the plants with a hypodermic syringe*

Smut culture	Four County White No. 97			Iodent No. 195			Iodent No. 224			Black 's Yellow Dent No. 344			Walden 's Yellow Dent No. 452			Golden Bantam Sweet No. 6883		
	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls
A1	12	75	53	10	60	50	20	85	86	26	57	43	17	70	65	24	92	83
A2	14	92	50	13	77	61	20	76	24	25	84	16	18	94	83	25	60	60
A3	15	0	0	10	40	0	22	23	0	25	12	0	18	50	0	26	23	8
A4	15	27	0	13	8	0	22	55	9	26	46	11	21	62	19	27	70	18
A5	15	33	0	7	0	0	22	82	50	27	74	37	22	77	50	28	89	89
Check ...	15	0	0	7	0	0	24	0	0	28	0	0	21	0	0	27	0	0

TABLE 7.—*Results of inoculating six inbred lines of corn with five cultures from the same smut gall by dropping the inoculum into the tops of the plants*

Smut culture	Four County White No. 97			Iodent No. 195			Iodent No. 224			Black's Yellow Dent No. 344			Walden's Yellow Dent No. 452			Golden Bantam Sweet No. 6883		
	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls
A1	20	25	5	23	22	22	24	16	13	17	53	41	21	48	28	15	0	0
A2	17	58	0	21	14	14	25	12	0	22	36	0	27	66	4	23	0	0
A3	12	50	0	19	26	12	16	0	0	24	33	4	24	66	0	19	0	0
A4	16	62	0	25	44	44	25	40	20	21	62	38	17	89	0	22	5	5
A5	13	61	30	26	38	35	24	17	0	18	33	33	26	73	57	22	9	9
Check ...	20	0	0	17	0	0	25	0	0	23	0	0	22	0	0	21	0	0

TABLE 8.—*Summary showing lines of corn infected with smut when hypodermically inoculated with five cultures of U. zeae from the same smut gall, and by dropping the inoculum into the tops of the plants*

Method of inoculation	Number of lines inoculated	Number lines infected when inoculated with									
		A1		A2		A3		A4		A5	
		Nec.[a] Clo.	Galls present	Nec. Clo.	Galls present	Nec. Clo.	Galls present	Nec. Clo.	Galls present	Nec. Clo.	Galls present
Inoculum forced into young tissue hypodermically	6	6	6	6	6	5	1	6	4	5	4
Inoculum dropped on apical buds	6	5	5	5	2	4	2	6	4	6	5

[a] Nec., necrotic; Clo., chlorotic.

TABLE 9.—*Results of inoculating five inbred lines of corn hypodermically with monosporidial cultures of Ustilago*

TABLE 9.—*Results of inoculating five inbred lines of corn hypodermically with monosporidial cultures of Ustilago zeae and with different combinations of these cultures*

Smut culture[a]	Iodent No. 224a			Krizer Bros. Yellow Dent No. 379			C. I. No. 204 (273)			Lancaster Sure Crop No. 311			Clark's Yellow Dent No. 440a		
	Number plants inoculated	Number plants infected	Number plants with galls	Number plants inoculated	Number plants infected	Number plants with galls	Number plants inoculated	Number plants infected	Number plants with galls	Number plants inoculated	Number plants infected	Number plants with galls	Number plants inoculated	Number plants infected	Number plants with galls
Wis. 1	30	10	0	19	17	0	28	6	0	5	0	0	10	5	0
Arl. Farm 2	26	7	0	22	7	0	34	5	0	7	0	0	9	7	0
Mich. 10	29	19	0	12	11	0	30	11	0	6	1	0	11	3	0
Arl. Farm 14	30	4	0	17	10	0	36	3	0	6	0	0	10	6	0
S. Dak. 37	7	3	3	—	—	—	—	—	—	5	2	1	10	7	3
Kan. 38	28	13	0	21	12	0	32	5	0	7	5	0	10	6	0
Wis. 1 + Arl. Farm 2	32	4	0	22	10	0	34	6	0	1	0	0	10	5	0
Wis. 1 + Mich. 10	27	23	7	11	10	10	34	9	0	2	1	0	9	6	0
Wis. 1 + Arl. Farm 14	30	6	0	15	0	0	33	0	0	5	3	0	9	3	0
Wis. 1 + S. Dak. 37	9	7	4	—	—	—	—	—	—	3	3	0	10	3	3
Wis. 1 + Kan. 38	32	25	17	15	14	14	26	13	6	2	0	0	9	7	5
Arl. Farm 2 + Mich. 10	31	24	6	17	17	11	33	21	4	8	6	0	10	5	3
Arl. Farm 2 + Arl. Farm 14	28	27	6	13	12	2	27	9	0	5	5	0	10	7	0
Arl. Farm 2 + S. Dak. 37	7	6	6	—	—	—	—	—	—	5	5	0	8	7	5
Arl. Farm 2 + Kan. 38	25	19	6	15	14	8	28	15	1	6	2	2	10	8	6
Mich. 10 + Arl. Farm 14	31	23	0	24	0	0	30	1	0	4	0	0	9	2	0
Mich. 10 + S. Dak. 37	6	5	5	—	—	—	—	—	—	5	2	2	9	8	4
Mich. 10 + Kan. 38	27	8	0	15	0	0	24	2	0	4	0	0	10	7	0
Arl. Farm 14 + S. Dak. 37	10	7	5	—	—	—	—	—	—	5	2	2	10	8	3
Arl. Farm 14 + Kan. 38	32	5	0	13	0	0	26	1	0	4	3	0	10	6	0
S. Dak. 37 + Kan. 38	8	5	5	—	—	—	—	—	—	5	4	4	10	7	4
Check	30	0	0	23	0	0	28	0	0	6	0	0	10	0	0

[a] Wis., Wisconsin; Arl. Farm, Arlington Farm; Mich., Michigan; S. Dak., South Dakota; Kan., Kansas.

tissue are homothallic and arise through mutation. However, Maire's (25) studies on the nuclear behavior of *U. zeae* suggests that homothallic cultures may arise in another manner. Maire found that when a chlamydospore of *U. zeae* germinated, its nucleus passed into the promycelium and divided. Some times the daughter nuclei did not divide, at other times only one divided, while, normally, both daughter nuclei divided. Upon germination chlamydospores formed promycelia consisting either of two, three, or four cells, each cell of which contained one nucleus. The nucleus of each cell divided when a sporidium was budded off and one of the daughter nuclei passed into this sporidium. If it is assumed that the first division of the nucleus in the promycelium is equational and the second reductional, sporidia budded off from a promycelial cell whose nucleus has undergone only one division might possess a nucleus in the diploid condition. Hanna (18) stated that segregation of factors may occur at both divisions of the spore nucleus. A sporidium budded off from a promycelial cell whose nucleus has undergone two divisions, before the final one which produces a daughter nucleus that passes into the sporidium, would possess a nucleus in the haploid condition. Thus it is possible that monosporidial cultures of *U. zeae*, such as South Dakota 37, that produce chlamydospores in host tissue, are homothallic, and are in a diploid condition if they originate from sporidia borne on a promycelial cell with an unreduced nucleus. Such diploid sporidia, theoretically, could be borne only by two or three-cell promycelia. Monosporidial cultures which do not produce chlamydospores in host tissue must be in a haploid condition and originate from sporidia borne on cells of promycelia whose nuclei have undergone reduction division. The data in table 9 show that certain monosporidial cultures have to be paired to produce chlamydospores in corn plants (10, 18).

TABLE 10.—*All possible pairings of five monosporidial cultures of Ustilago zeae*

		A[a]	B	C		D
		Wis. 1	Arl. Farm	Mich. 10	Kan. 38	Arl. Farm 14
A	Wis. 1	–[b]	–	+	+	–
B	Arl. Farm 2	–	–	+	+	+
C	Mich. 10	+	+	–	–	–
	Kan. 38	+	+	–	–	–
D	Arl. Farm 14	–	+	–	–	–

[a] A, B, C, and D sexual strains.

[b] – No chlamydospores formed in host tissue; + chlamydospores formed.

When the five monosporidial cultures were paired, as shown in table 10, mating occurred between certain pairs which produced chlamydospores in host tissue. This indicates the presence of sex strains in *U. zeae*. It has been demonstrated that such strains occur in several genera of the Basidiomycetes, including the genus Ustilago. Theories based on the presence of two and more than two sex strains have been used to explain the sexual reactions of these fungi.

The bisexuality theory explained the sexual behavior of *Aleurodiscus polygonius* (Pers.) Hoehnel and Litsch (23), *Coprinus rostrupianus* Hansen (28) and *C. radians* (Desm.) Fr. (43). The same theory failed to explain the sexual nature of *C. lagopus* Fr. and *C. niveus* (Pers.) Fr. (27), and Hanna (17) proved that four sex strains were present in *C. lagopus*. Sass (35) has shown that the presence of one pair of Mendelian factors does not determine the sex of paired mycelia of *C. ephemerus* (Bull.) Fr.

Kniep (22) noted sexual reactions among conjugating germ tubes of several different species of Ustilago. Dickinson (7, 8) found that there were only two sexes in *Ustilago levis* and two in *U. hordei*, and Stakman (37) concluded from his work with paired multisporidial cultures of *U. zeae* that the presence of two sexes in this fungus explained his results.

The results obtained from mating the five monosporidial cultures of *U. zeae*, referred to in table 10, indicated that the cultures belonged to four sexual groups or strains (10). Michigan 10 and Kansas 38 behaved as if they belonged to the same sex strain, and the other cultures, Wisconsin 1, Arlington Farm 2, and Arlington Farm 14, behaved as if they belonged to each of three other sex strains. The four sex strains to which the five cultures belonged were designated A, B, C and D. Chlamydospores were produced in host tissue when A was paired with C but not when paired with B and D. Chlamydospores also were produced when B was paired with C and D. No chlamydospores were formed when C was paired with D.

Pathogenicity of multisporidial cultures and their combinations. A test similar to the preceding one with monosporidial cultures was made to study the behavior of three multisporidial cultures and their different combinations. Iowa cultures 1, 5, and 7 were selected for this study, for, as indicated by their behavior, they had apparently become weakly pathogenic (Table 3). The data in table 11 show that multisporidial cultures 1, 5, and 7 produced no galls. Their behavior was similar to that of the five monosporidial cultures reported in the preceding test. However, cultures 1 and 5 had previously formed galls on plants in the greenhouse (Table 3). Culture 7 had produced galls on one inbred line (Table 4). In table 11 it is shown that culture 1 + 7 and 5 + 7 produced galls and 1 + 5 did not. The interaction of the proper sex factors may have been responsible for the production of galls by the first two combinations, and the inability of the

TABLE 11.—*Virulence of Iowa cultures 1, 5, and 7 and their combinations on four inbred lines of corn inoculated hypodermically*

Smut culture	Four County White No. 5			Iodent No. 224a			Iodent No. 238			Walden's Yellow Dent No. 460		
	Number plants inoculated	Number plants infected	Number plants with galls	Number plants inoculated	Number plants infected	Number plants with galls	Number plants inoculated	Number plants infected	Number plants with galls	Number plants inoculated	Number plants infected	Number plants with galls
1	9	0	0	31	0	0	26	24	0	19	0	0
5	10	0	0	31	7	0	27	23	0	19	3	0
7	8	0	0	29	20	0	28	27	0	16	8	0
1+5	9	0	0	27	7	0	28	22	0	16	1	0
1+7	9	7	4	28	12	0	26	23	16	14	1	0
5+7	9	6	5	30	14	4	25	19	11	19	3	1
Check ...	3	0	0	60	0	0	28	0	0	3	0	0

third combination to form galls may have been due either to the absence of some essential factor or factors or to the presence of lethal agents.

Effect of repeated transfers on the virulence of cultures of U. zeae. Three inbred lines of corn were inoculated hypodermically with four pairs of multisporidial cultures of *U. zeae* from Iowa. Each pair came from the same stock culture. One member of the pair had been transferred only once from the date of its isolation and the other six times. The object of the test was to determine whether repeated transfers to carrot agar, as compared to one transfer, would affect the virulence of the cultures.

Iowa 1, 4, and 6, transferred once, produced galls, as is shown in table 12. Iowa 7 transferred once, did not form galls. None of the cultures produced galls after they had been transferred six times. Loss of virulence has been reported as occurring, also, in pure cultures of *Fusarium conglutinans* Woll. (13). Since the four pairs of cultures of *Ustilago zeae* were of multisporidial origin, it is probable that when isolated, each contained sporidia of at least two sexual groups. When injected into corn plants shortly after isolation, galls were formed by all cultures except Iowa 7. At the end of six transfers of cultures 1, 4, and 6, one or more of the essential sexual groups might have been lost, and the cultures no longer produced galls on the inoculated plants. In the case of Iowa 7 the essential sexual factors probably were lost at the first transfer.

Virulence of conidia of U. zeae exposed to winter temperature. A test-tube culture of *U. zeae* on carrot agar was put in a loosely covered Mason fruit jar and placed in an exposed place on the north side of the greenhouse.

TABLE 12.—*Comparison of the virulence of four Iowa cultures transferred only once and transferred six times during a six months' period*

Inbred line of corn	Iowa 1						Iowa 4						Iowa 6						Iowa 7					
	Transferred						Transferred						Transferred						Transferred					
	One time			Six times			One time			Six times			One time			Six times			One time			Six times		
	Number plants inoculated	Number plants infected	Number plants with galls	Number plants inoculated	Number plants infected	Number plants with galls	Number plants inoculated	Number plants infected	Number plants with galls	Number plants inoculated	Number plants infected	Number plants with galls	Number plants inoculated	Number plants infected	Number plants with galls	Number plants inoculated	Number plants infected	Number plants with galls	Number plants inoculated	Number plants infected	Number plants with galls	Number plants inoculated	Number plants infected	Number plants with galls
Iodent No. 238	27	23	18	26	14	0	27	19	4	26	24	0	27	24	8	28	21	0	28	0	0	27	22	0
Reid's Yellow Dent Proudfit's Strain No. 358	22	18	18	19	7	0	21	2	0	21	4	0	21	16	12	19	12	0	20	19	0	21	11	0
Clark's Yellow Dent No. 440a	19	6	3	21	0	0	21	16	1	21	9	0	20	3	3	23	7	0	23	13	0	24	4	0

Another culture was placed in the refrigerator where stock cultures of various fungi were kept. The cultures used in this test were transferred on the same date from Iowa stock culture No. 8, a pure culture of smut isolated from a gall collected from a field at the Agronomy Farm at Ames. The test was started December 2, 1927, and on April 11, 1928, 131 days later, conidia from both cultures were transferred to sterile carrot decoction. Six days later, when the inoculum was ready, an equal number of plants from one inbred line of corn was inoculated with the two cultures.

The results reported in table 13 show that conidia growing on carrot

TABLE 13.—*Virulence of conidia of U. zeae kept in a refrigerator and exposed to outside winter temperature*

Inbred line of corn	Black's Yellow Dent No. 356	
Treatment of cultures	Culture kept outside	Culture kept in refrigerator
Number of plants inoculated	46	48
Number of plants infected	36	32
Number of plants with galls	25	7

agar, when exposed to outside winter temperature retain their power to infect corn seedlings. In fact, the culture kept outside appeared to be the more virulent of the two, as it produced galls on over three times as many plants as did the culture kept in the refrigerator. These results are comparable to those of Piemeisel (29) who found that sporidia were injured very little by freezing.

Comparative effectiveness of two methods of seedling inoculation. Inbred lines of corn were inoculated by dropping the inoculum on the apical buds of plants and by hypodermic injection of the inoculum into the young tissue. The relative effectiveness of the two methods of inoculation is illustrated in figures 3 and 4 in which are shown the results of inoculating inbred lines with different cultures of *U. zeae*.

It is quite evident that the hypodermic method of inoculation was the more effective in inducing smut, for, with only few exceptions, a higher percentage was obtained by this method than by dropping the inoculum on the buds of the plants. Conidia have to be in contact with susceptible host tissue for infection to occur. The conidia were placed nearer susceptible tissue when the inoculum was hypodermically injected than when dropped on the apical bud.

Relation of the condition of the apical buds to smut infection. Conidial suspensions were dropped into the tops of plants averaging about twelve

inches in height.[4] Apical buds were described as open, two-thirds open, one-third open, and closed, respectively, depending upon the size of the receptacle formed by the leaves at the top of the plant.

The results reported in table 14 show approximately twice as great a

TABLE 14.—*Relation of the condition of the apical buds of plants and percentage of plants smutted when seedlings were inoculated by the dropper method with conidial suspensions of U. zeae*

Condition of apical buds	Total number plants inoculated	Number of plants infected	Per cent plants infected	Number plants with galls	Per cent plants with galls
Open	568	222	39.0	88	15.5
Two-thirds open	2016	445	22.0	132	6.5
One-third open	698	85	12.2	14	2.0
Closed	136	12	8.8	1	.7

percentage of infected plants and plants with galls among the open plants as among those that were two-thirds open, and there was correspondingly less smut on plants with buds one-third open and buds closed. Apparently, open apical buds hold the inoculum and allow conidial suspensions to seep down in the plants and come in contact with young, actively growing and susceptible tissue (2). Inoculum seldom reaches susceptible tissue in plants with closed buds.

FIELD EXPERIMENTS

Field tests of the comparative pathogenicity of certain smut cultures were made during the summer of 1928 at the Florida Agricultural Experiment Station, Gainesville, Florida. The corn for these tests was planted on May 19 and was inoculated at each of two different stages of its growth with inoculum from cultures of *U. zeae* that had proved pathogenic on seedlings in the greenhouse.

The plants inoculated hypodermically the first time were in the three- to four-leaf stage and from five to six inches tall. The inoculum was forced in until it ran out at the tops of the plants. At the time of making the second inoculations the plants ranged in height from one to two and a half feet. An attempt was made to insert the hypodermic needle in the stalk so as to hit the growing point, and 2 cc. of inoculum were forced into each plant.

[4] G. A. Platz, a former graduate student at Iowa State College, has a paper in press dealing with investigations on the structure of the corn plant in relation to its susceptibility to smut.

On examination the growing point was found to be located one to three inches above the soil in typical stalks.

Plants inoculated the first time by dropping the inoculum on the apical buds averaged about a foot in height. About 2 cc. of inoculum were dropped into each plant. The second inoculations by this method were made when the plants were from two to three and a half feet tall. Only a few plants had their tassels showing. In most cases, the tip of the developing tassel was several inches down in the stalk. Notes were taken on infection about two weeks from date of each inoculation, and three to four weeks intervened between the inoculations.

Plants under field conditions became chlorotic and necrotic when inoculated with sterile carrot decoction as well as those inoculated with the conidial suspensions of *U. zeae*. Even before any inoculations were made, a type of chlorosis and necrosis appeared on the leaves of plants scattered throughout the field. Undoubtedly, much of the chlorosis and necrosis of the tissues of inoculated plants was due to smut infection, for, in the greenhouse, only those plants inoculated with corn-smut conidia became chlorotic and necrotic. However, the uncertainty of being able to distinguish between chlorosis and necrosis caused by *U. zeae* and that due to other causes made it necessary, in the field, to use only the appearance of galls on the plants as a true symptom of smut infection.

Pathogenicity of three cultures of U. zeae from one smut gall. The results reported in table 15 show that no smut galls developed on plants of any of the lines hypodermically inoculated on June 2 with conidial suspensions of multisporidial cultures A1, A2, and A3 derived from the same smut gall. These results differ considerably from those obtained in the greenhouse where plants, at the same stage of growth, developed galls when inoculated with conidia from the above cultures. However, it should be noted that only two of the inbreds, Walden's Yellow Dent No. 452 and Golden Bantam Sweet No. 6883, used in the field, were inoculated with the same three cultures in the greenhouse. A1 and A2 formed galls on both of these lines in the greenhouse, and A3 produced galls on one of them (Table 6). Smut galls were produced on the five inbreds by the three cultures as a result of the inoculations made June 23. Even though there were relatively few plants in each of the lines inoculated, yet the results indicate that cultures A1, A2, and A3 differ in pathogenicity under field conditions. A type of smut infection obtained in the field as a result of the second inoculation is shown in figure 6.

Conidia injected into plants on June 2 possibly failed to come in contact with susceptible host tissue as no smut galls were formed. When the same plants were inoculated at or near their growing points on June 23 galls were produced. It is possible, also, either that environmental conditions

TABLE 15.—*Results of inoculating five inbred lines of corn hypodermically, in the field, on June 2 and 23, with three cultures of Ustilago zeae derived from the same smut gall*

Smut culture	C. I. No. 133 (134)						Walden's Yellow Dent No. 452						Clark's Yellow Dent No. 440						Lancaster Sure Crop No. 311						Golden Bantam Sweet No. 6883					
	6–2–28			6–23–28			6–2–28			6–23–28			6–2–28			6–23–28			6–2–28			6–23–28			6–2–28			6–23–28		
	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls
A1	34	0	0	31	7	23	10	0	0	8	2	25	3	0	0	2	0	0	33	0	0	24	16	66	32	0	0	29	1	3
A2	33	0	0	32	16	50	14	0	0	15	8	53	7	0	0	4	0	0	27	0	0	30	10	33	35	0	0	29	8	28
A3	11	0	0	8	6	75	16	0	0	15	11	73	1	0	0	1	0	0	34	0	0	24	1	4	26	0	0	26	2	8
Check ..	21	0	0	17	2	12	16	0	0	9	3	30	7	0	0	6	0	0	30	0	0	26	0	0	31	0	0	27	0	0

FIG. 6. Plant showing leaf and sheath infection as a result of hypodermic inoculation in the field.

were unfavorable for infection to occur from inoculations of June 2 or that the conidia did not possess the proper sexual factors necessary for gall production.

Smut galls that appeared on plants of the four inbreds, inoculated with conidia suspensions of cultures A1, A2, and A3, as is shown in table 16, were due to natural infection with the possible exception of C. I. No. 133 (134) inoculated with conidia from the A3 culture. Unfavorable environmental conditions in the field and the fact that conidia were not placed in direct contact with susceptible tissue are probably the explanation for the failure of the cultures to produce galls.

Pathogenicity of multisporidial and monosporidial cultures of U. zeae and their various combinations. Iodent No. 224a was inoculated hypodermically at two different dates with multisporidial and monosporidial cultures and their various combinations. Inoculations made on the first date showed that sporidial cultures used singly failed to produce galls and

TABLE 16.—*Results of inoculating five inbreds, in the field, on June 9 and July 2, with three smut cultures by dropping the inoculum on the apical buds of the plants*

Smut culture	C. I. No. 133 (134)						Walden's Yellow Dent No. 452						Iodent No. 195						Black's Yellow Dent No. 344						Golden Bantam Sweet No. 6883					
	6–9–28			7–2–28			6–9–28			7–2–28			6–9–28			7–2–28			6–9–28			7–2–28			6–9–28			7–2–28		
	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls
A1	12	0	0	6	1	16	19	0	0	13	3	23	23	0	0	21	3	14	29	0	0	20	0	0	29	0	0	22	5	23
A2	5	0	0	4	1	25	15	0	0	11	2	18	32	0	0	33	6	18	25	0	0	17	0	0	34	0	0	30	5	17
A3	17	0	0	14	8	57	21	2	9	18	4	22	28	0	0	28	2	7	31	1	3	29	4	14	29	0	0	26	4	15
Check ...	17	0	0	12	4	33	26	1	4	20	3	15	30	2	7	29	5	17	27	0	0	20	3	15	28	0	0	25	4	16

that only four combinations proved to be gall formers. These data are shown in table 17. The results of the second inoculations show that multi-

TABLE 17.—*Results of inoculating plants hypodermically, in the field, with conidial suspensions of multisporidial and monosporidial cultures and their various combinations*

Smut culture[a]	Iodent No. 224a					
	6-2-28			6-23-28		
	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls
Iowa 1	10	0	0	9	8	88
Iowa 5	19	0	0	11	5	45
Iowa 7	14	0	0	14	0	0
Wisconsin 1	14	0	0	14	2	14
Kansas 38	13	0	0	14	4	29
Iowa 1 + Iowa 5	19	4	21	15	13	87
Iowa 1 + Iowa 7	16	0	0	15	14	93
Iowa 5 + Iowa 7	19	1	5	16	15	94
Wisconsin 1 + Iowa 1	17	0	0	17	14	82
Wisconsin 1 + Iowa 5	16	0	0	16	3	19
Wisconsin 1 + Iowa 7	10	0	0	10	6	60
Kansas 38 + Iowa 1	18	4	22	15	14	93
Kansas 38 + Iowa 5	18	0	0	15	14	93
Kansas 38 + Iowa 7	13	0	0	14	1	7
Wisconsin 1 + Kansas 38	17	7	41	16	7	44
Check	19	0	0	17	3	18

[a] Iowa 1, 5, and 7, multisporidial cultures; Wisconsin 1 and Kansas 38, monosporidial cultures.

sporidial cultures, Iowa 1 and Iowa 5, are evidently gall formers, as many more plants had galls when inoculated with these two cultures than did the check plants on which galls appeared as the result of natural infection. Iowa 7 did not form galls and the results do not signify that Wisconsin 1 and Kansas 38 are gall producers on Iodent No. 224a.

The results obtained under field conditions are similar to those obtained in the greenhouse, as the combination cultures were more virulent than the single cultures comprising them. This is illustrated by Iowa 1, 5, and 7 used singly and in different combinations with each other.

CULTURAL STUDIES

A comparison of the cultural characteristics of different cultures of *U. zeae* used in the preceding pathogenicity studies was made by growing

TABLE 18.—*Cultural characters of twelve cultures of Ustilago zeae, from different localities in Iowa, when grown under the same conditions on carrot agar in Erlenmeyer flasks*

TABLE 18.—*Cultural characters of twelve cultures of Ustilago zeae, from different localities in Iowa, when grown under the same conditions on carrot agar in Erlenmeyer flasks*

Culture	Age in days	Dimensions of colonies mm.	Color	Topography	Surface	Amount of aerial mycelium	Zonation	Margin
1	69	36 x 59	Brick red center with light purple ring grading to white at margin	Raised center, rope like; sectors present	Waxy center; cottony border	Medium	Yes	Filamentous
2	69	24 x 33	Purple to light brown center with white sectors	Raised center, slightly wrinkled to smooth, radially rayed; sectors present	Flaky to waxy and powdery	None	do	Not filamentous
3	69	23 x 27	Light brown with purple center	Raised wrinkled center, pitted slightly; sectors present	Dull waxy to ashen	Slight	do	Filamentous
4	69	30 x 37	Light brown to purple center grading to white at border	Raised center, pitted and wrinkled with few radial rays; sectors present	Dull waxy to cottony	do	do	do
5	69	28 x 44	Brownish purple center with white border	Raised center, rayed and wrinkled with deep furrows, smooth at border; sectors present	Waxy to cottony	Medium	do	do
6	69	42 x 42	Light purple center with white center ring	Raised center; sectors present	Cottony to velvety	Abundant	Moderate at margin only	do
7	69	37 x 39	White center with purple ring	Raised center, furrowed and slightly pitted, radially rayed	Waxy to velvety	do	None	do
8	69	40 x 45	White to purple throughout	Center raised and wrinkled, radially rayed	Waxy to cottony	Medium	do	do
9	69	31 x 32	Tan center with chalk white and purple sectors	Raised center, wrinkled; sectors present	Waxy to velvety	Slight	Yes	do
10	69	30 x 43	Purple center with tan ring; white and purple in sectors	Wrinkled and pitted center with smooth sectors	Waxy to velvety and powdery	do	do	Slightly filamentous
11	69	29 x 37	Deep purple center grading to brown with white border	Raised center and ridged at border; sectors present	Waxy to cottony at border	do	do	Filamentous
12	69	39 x 45	Tan to purple throughout	Raised center and much wrinkled to border; sectors present	Waxy to slightly powdery	Medium	do	do

them on 20 cc. of carrot agar in 200 cc. Erlenmeyer flasks. The carrot agar used in culturing each group of cultures was prepared and sterilized at the same time. After inoculation the flasks were kept at room temperature in the same locker in the laboratory so that they would be exposed to the same environmental conditions.

The data on the cultural characters of Iowa cultures 1 to 12, inclusive, at the start of the pathogenicity test previously reported in this paper, are summarized in table 18. These data are descriptive of the cultures grown on carrot agar at the age of 69 days. The colonies of the cultures differed in color, rate of growth, etc. Sectors when present varied in size and shape. No two cultures possessed identical characters. These results are comparable to those of Christensen and Stakman (3) who found that physiologic forms of *U. zeae* could be distinguished in culture. Further studies showed that colonies of the same culture, grown on carrot agar in separate Erlenmeyer flasks, for the same length of time and under the same environmental conditions, in some cases closely resembled each other and in other cases appeared to be different. Cultures 6, 8, and 12 were the most pathogenic of the twelve, and cultures 1, 5, and 7 were the least pathogenic. Their cultural characteristics do not show that any particular character or group of characters could be used as a criterion for the identification of cultures differing in pathogenicity.

Cultures A1 to A5, inclusive, derived from the same smut gall, differed from each other in their cultural characteristics, and some of these differences are noticeable in figure 7. The six monosporidial cultures differed as

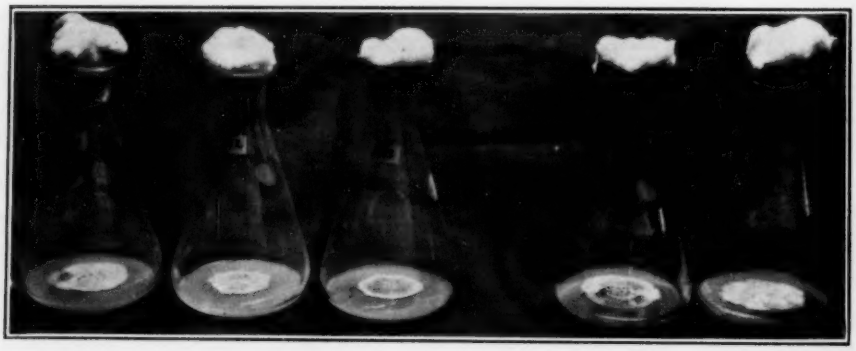

FIG. 7. Cultures A1 to A5 from the same smut gall growing on carrot agar at the end of twenty-five days.

greatly from each other in their cultural characters as did any of the multisporidial cultures used in these studies.

DISCUSSION

The conclusions regarding the relative pathogenicity of the cultures of *U. zeae* used in the present investigations were based mainly on the ability of the cultures to produce smut galls on the different inbred lines which served as differential hosts. Exact differences that exist in the pathogenicity of different cultures of the fungus can be ascertained only through a statistical analysis of the data collected. To determine such differences will require the use of larger numbers of plants of each inbred line for inoculation than were used in testing the pathogenicity of the various cultures of *U. zeae* used in the present studies.

Hanna (18) reported that Mendelian factors in *U. zeae* control its sexual behavior and the expression of its pathogenicity. Researches (25, 30, 36) on the nuclear behavior of *U. zeae* indicate that a nuclear mechanism exists for the segregation and interaction of genetic factors. Seyfert (36) stated that clamp connections occurred in the vegetative mycelium of *U. zeae* and that the binucleate condition arose sometime before spore formation. Rawitscher (30) has reported that sporidia are uninucleate and give rise to uninucleate mycelia in the host plant. The single nucleated hyphal cells fuse and give rise to binucleate cells which finally become mature chlamydospores with one nucleus. Maire (25) found that the nucleus of a germinating chlamydospore always divided once and generally twice in the promycelium before sporidia were budded off. As thus outlined, the life cycle of the organism involves a nuclear fusion to form the chlamydospore and a nuclear reduction to form the sporidia. Such a nuclear mechanism forms a basis for the assumption that sexual factors controlling the degree of pathogenicity exist in *U. zeae* and are segregated out at reduction division and combined in different ways at nuclear fusion. Cultural differences that were noted between cultures grown on the same media probably were due partly to mutation (3, 39) and partly to the expression of different genetic factors carried by the cultures. Furthermore, it is possible that the physiologic reactions of the fungus are very delicately balanced and that only a slight difference in water content, acidity, etc., of the culture media was responsible for some of the differences in cultural characteristics.

SUMMARY

All multisporidial cultures of *Ustilago zeae*, including the twelve cultures derived from smut galls from different localities in Iowa, and the five cultures isolated from one smut gall, differed from each other in their pathogenicity on inbred lines of corn.

Ustilago zeae is regularly heterothallic and one monosporidial culture behaved as if homothallism also occurs in the organism. When five mono-

sporidial cultures were mated, they were found to represent four sex strains of *U. zeae*.

Paired multisporidial cultures were generally more virulent than the individual cultures comprising them.

Conidia of *U. zeae* retained their ability to produce smut when exposed to winter temperature, and cultures of the organism lost their ability to produce smut galls when repeatedly transferred.

Hypodermic injection of the inoculum into young tissues of corn plants resulted in a higher percentage of smut infection than did dropping the inoculum on the apical buds. When the latter method was used in the greenhouse, more smut was produced on plants with open apical buds than on those with closed buds. Both methods of inoculation gave a higher percentage of smut infection in the greenhouse than in the field.

Colonies of *U. zeae* from the same and different cultures differed from each other in their cultural characteristics.

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HOST RANGE AND LIFE-HISTORY STUDIES OF SOME LEGUMINOUS ASCOCHYTAE[1]

RODERICK SPRAGUE

INTRODUCTION

Leguminous plants are subject to attack by many named species of Ascochyta. Recent work (9), (10), (12), (13) makes clear the need of further study of these fungi as a group. On peas (*Pisum sativum* L.), Ascochytae, originally comprised in one species (*Mycosphaerella pinodes* (Berk. and Blox.) Stone), were made into two species with *Ascochyta pisi* Lib. distinct from *M. pinodes* which was shown to have a sphaeropsidaceous stage of its own. In addition, a third type on pea, lately made a species *A. pinodella* L. K. Jones (10), was described (13). The difference in the pathogenicity and life histories of these three pea-invading organisms is of considerable importance to the grower and to the pathologist and, since literature is replete with the citations of *A. pisi* on a wide range of hosts, it is desirous to know these fungi's true nature.

The primary purpose of this paper therefore is (1) to present to the reader an account of the life histories and pathological capabilities of present-known species of Ascochyta on legumes, (2) to list their known hosts and to make revisions in species in accordance with all information brought forth. This paper will deal principally with Ascochyta species on the following naturally infected hosts:

1. *Vicia villosa* Roth.
2. *Vicia sativa* L.
3. *V. angustifolia* (L.)Reich.
4. *Lathyrus odoratus* L.
5. *Lathyrus tingitanus* L.
6. *Vicia faba* L.
7. *Medicago sativa* L.
8. *Medicago lupulina* L.
9. *Phaseolus spp.*
10. *Pisum sativum* L.

METHODS AND PROCEDURE

In keeping with the needs of the pathologist who is dealing with living organisms the writer has here attempted to cover not only morphological but physiological characteristics of the organism under consideration. He has employed standard, easily duplicatable methods that would tend to throw light on the host ranges and life histories of the types involved. The investigation was conducted along the following lines of procedure:

1. With the aid of the microscope all available herbarium material of Ascochyta in the United States and, in addition, some foreign material were studied.

[1] The writer wishes to thank Drs. O. T. Wilson, J. J. Davis, F. R. Jones, and M. B. Linford for material help and advice throughout the course of the work. He is indebted to many for herbarium material.

2. Study of personal collections and of forms in the field.

3. Camera lucida drawings and spore measurements (50 spores from a collection) were made of all material seen.

4. Isolation of Ascochyta from various hosts, from pod, leaf, stem, and hypocotyl and maintenance of cultures in sporulating condition.

5. Study of cultures of Ascochyta on various media, the most valuable of which were potato-1 per cent dextrose agar (13), and pea-soup agar.[2]

6. Inoculation studies of extensive nature on aerial and subterranean parts.

7. Attempts to develop ascigerous stages.

One of the first problems in a work of this kind is to determine just what factors, characters, and activities of the various fungi shall be employed in separating them into taxonomic divisions. The writer has used what he considers reliable mycological characters on host material as a basis, making use of both natural and, with due caution, artificial infections. He has also conducted cultural studies on artificial media in the determination of species. It is his opinion that, since fungi are so variable, it is not desirable to give technical names to varieties and forms, especially in this work where so many intermediate stages occur.

HISTORICAL

This paper, which deals primarily with available North American species, will omit mention of many familiar foreign hosts and Ascochyta species.

Types on Vicia species. Libert (11) describes *Ascochyta viciae* on *Vicia sepium* as the cause of a subrotund spot with a darker margin than the paler interior and showing epiphyllous, aggregated pycnidia (90–100 μ in diameter) containing hyaline 1-septate spores 12–15 x 4.5 μ. The description is very similar to that for *A. pisi* (13).

Cooke (3, p. 115) attempted to place Libert's specimen under his *Phyllosticta viciae* but seems to have been concerned with an immature Ascochyta.

Trail (29) describes *A. vicicola* (Trail) Sacc. on *V. sepium* from Scotland and, except for its slenderer spores (13–16 x 2.5–3 μ), the description sounds similar to *A. viciae* Lib.

Sydow (27), (28) describes *A. vicia-lathyroidis* from Germany. He also names *A. ervicola* on *Vicia hirsuta* S. F. Gray.

Spegazzini (23, p. 321) describes a dark yellowish to brown leaf spot on *Vicia faba* from Argentine. His species *A. fabae* is characterized by large spores (10–25 x 5–6 μ).

[2] Pea soup agar was made by adding the contents of an 8 ounce can of Campbell's pea soup to 2 liters of distilled water, solidified with 1.3% agar, filtered, tubed, plugged, and sterilized at 15 lbs. for twenty minutes.

Among extensive and more recent work we find Stone (24) including both *A. viciae* Lib. and *A. vicicola* under *Mycosphaerella pinodes* and Scalia (22), even more sweeping, in grouping practically all the forms on peas, vetches, and beans under *A. pisi* Lib.

Recently, it was pointed out by Linford and Sprague (13) that an Ascochyta spot was of common occurrence on *Vicia angustifolia* in Wisconsin and that hosts and culture symptoms (potato-2 per cent dextrose agar) bore close resemblance to *A. pisi* and none to cultures of *M. pinodes*.

Both Stone (24) and Atkinson (1) found reason to conclude *M. pinodes* occurred on vetch in that an ascigerous stage identical to *M. pinodes* was found on hairy vetch.

Types on Lathyrus species. Trail (29) described *Ascochyta lathyri* on *Lathyrus silvestris* as causing an ill-defined, often extensive leaf spot bearing numerous small pycnidia that contained spores measuring 8–10 x 2.5 μ. Saccardo (19) notes them still smaller in his work, 5–8 x 2.3 μ.

Stone (25) in describing *Mycosphaerella ontarionenis* stresses the small size of the pycnidiospores.

A large-spore Ascochyta, *A. orobi*, has been described on *Lathyrus vernus* by Saccardo (18, p. 303). Harkness (7) gives *A. pisi* on the leaves of *L. palustris* from California.

Bubák and Kabát (2) described *A. lathyri* var. *lathyri-odorati* as the cause of light colored to brownish spots of a general, indefinite nature on the leaves of the sweet pea. Spores were small, 8–14 x 2.5–3.5 μ.

Types on Medicago species. Ascochyta imperfecta Pk. (14), (26), (4) is considered the chief Ascochyta species on alfalfa in this country. It differs from *A. medicaginis* Bres. in smaller, frequently non-septate, spores.

Rostrup (17) from Denmark has mentioned *A. pisi* on alfalfa and Saccardo (20) describes *A. pisi* var. *medicaginis* on living stems of alfalfa from Spokane, Washington.

SYMPTOMATOLOGY

Types on Vicia species. Without exception leaf-, stem-, and pod-spot material on *Vicia villosa, V. sativa,* and *V. angustifolia* showed light buff to white spots (4–10 mm. diameter), definitely red or brown-bordered and having prominent pycnidia usually concentrically zoned.

On *Vicia faba* the type seen (leaf spot from California) appeared as large spots, light-centered with a tendency towards shot-holing and bordered by a discolored, black, sometimes red border. The large pycnidia were prominently arranged in concentric order. Infected seed of this host showed sunken brown to black spots up to a centimeter in diameter.

Types on Lathyrus species. Most of the work was done with material on sweet peas (*L. odoratus*). Material from Minnesota showed vines cov-

ered with purple to brown stem lesions in which many black-brown small pycnidia occurred. Leaf spots were vague, indefinite light brown to pale tan in color.

Types on Medicago species. On alfalfa (*Medicago sativa*) injury is (except for material collected by Simmons in Wyoming and Washington) confined to the sides and tips of leaves and petioles as indefinite diffused brown bordered spots with lighter centers and obscure pycnidia.

On *M. lupulina* spots are very light with narrow but definite borders and large pycnidia, fairly prominent.

ETIOLOGY

Types on Vicia species. Because of the confusion relative to this group it seems well to list a considerable number of specimens with brief notes where it is deemed advisable. Measurements are of mean spore size when not otherwise given.

Vicia villosa Roth.

J. M. Bates (U. S. D. A.), Nebr., 1897. Spores broader proportionately than most of the collections to follow, 14.6 x 4.3 μ; Barth.: Fungi Columb. 1506, Alabama, 1901, pod spot, 14.6 x 4.1 μ from specimen at Ohio State Univ., 13.7 μ x 3.2 μ from specimen at Wash. State College; Barth.: Fungi Columb. 2305. (The host *Vicia americana* Muhl. seems to be *Vicia villosa* Roth. from material in the Bureau of Plant Industry Herbarium at Washington, D. C., 13.7 x 4.3 μ; Clinton, Mass., 1906, spores cylindrical with homogenous contents, 12.7 x 3.9 μ; Clinton, Granby, Conn., 1907, 13.8 x 4 μ; J. J. Davis, Wis., 1914, 13.6 x 3.7 μ, spots seem immature with greenish, water-soaked appearance noted in stages of inoculated infection development; R. M. McKee (U. S. D. A.), Fayetteville, Ark., 1919, pycnidia 70–100, spores 13.2 x 3.6 μ; R. M. McKee, Georgia, 1921, 14.8 x 3.9 μ; J. H. Miller, Georgia, 1923, leaf spot, spores cylindrical, contents clear, hyaline 13.8 x 4 μ; Linford, N. J., 1928, R. F. Poole, N. J., 1924, pod spot, spores cylindrical, blunt ends, 13.6 x 3.6 μ; Kienholz, Pullman, Wash., 1927, leaf spot, spores, 12.5 x 4.3 μ, pod spot spores, 13.1 x 4.2 μ; Sprague, Ohio, 1928, 13.4 x 4.3 μ; Sprague, Ohio, 1928, 13.4 x 4.5 μ; Wolf, N. Y., spores slender, 12.9 x 3.4 μ; Vestergrun, Microm. rar. sel. 1537. Suecia, 1898, slender, cylindrical spores with homogeneous contents, 15.3 x3.7 μ.

Vicia sativa L.

Dearness, Ontario, 1897, spores clear, cylindrical, 15 x 4.5 μ, pycnidia 60–100 μ diameter; Tranzschel et S. Myc. Rossica 37, pod spot, pycnidia arranged concentrically, spores large, frequently several septate, similar to material from Mississippi, 16.6 x 4.7 μ; J. H. Miller, Ga.; L. E. Miles, Miss., 1922, 14.1 x 4.3 μ; H. H. Wedgeworth, Miss., 1927, spores large,

variable, cylindrical, somewhat bulgy, especially the frequent two or more septate ones, 16.1 x 4.6 μ (13–19 x 3.5–6 μ), pycnidial walls loosely structured, light golden, pycnidia of comparatively large size.

Vicia angustifolia (L.) Reich. and varieties.

H. M. Barre, Clemson College, S. C., 1909, 13.4 x 4.1 μ; Kabát et Bubák Fungi Imp. Ex. Ger. (Labelled *A. vicia-lathyroidis* Syd., 13.7 x 4.3 μ; R. M. McKee (U. S. D. A.), Ga., 1921, 15.4 x 4.1 μ, many are more than one-septate and range up to 22 μ in length; J. J. Davis, Barneveld, Wis., 1921, spores cylindrical and not constricted in some, strongly so in others, 14.2 x 4.2 μ; Davis, Ridgeway, Wis., 1921, spores cylindrical, 13.4 x 4.2 μ; L. E. Miles, Miss., 1922, 13 x 4.5 μ; J. H. Miller, Ga., 1923, 15.6 x 4.1 μ; Linford, Wis., 1924, spores cylindrical, some two-septate, 15 x 4 μ, in culture on potato-2 per cent dextrose agar this resembled *A. pisi* from the pea.

Vicia faba L.

F. R. Jones, Corvallis, Oregon, 20.3 x 4.3 μ; R. M. McKee, Chico, Calif., 17.3 x 4.7 μ.

While great variation in size may be found both in spores and pycnidia, even in the same collections, those of the Ascochyta on vetches range distinctly larger in size over those of *A. pisi* on the pea. The smallest are probably those on *V. villosa* and all transitions occur between these and the large type on *V. faba*. Under the oil-immersion lens the pycnidia of the latter are light golden in color and show a complex of intertwining mycelium which is not quite so compact as that of the firmly knit pycnidial stage of *M. pinodes* on pea. There is a tendency away from the formation of an outer wall marked off in squares or polygons and the ostiole is less definite.

Types on Lathyrus species. Spores of material collected by Vande Vere from Hopkins, Minnesota, on *L. odoratus* were small, plump, cylindrical to faintly fusiform and had a mean spore size of 9.4 x 3.6 μ (Pl. XXXV, I).

Infected pod material of *L. odoratus*, sent by F. R. Jones from Madison, Wis., showed spores and pycnidia identical to *M. pinodes* on one pod but on the others of the collection the material was indistinguishable from the Minnesota collection.

Types on Medicago species. Material examined from eleven widely scattered States show the spores of *A. imperfecta* Pk. on alfalfa to average 10.6 x 2.8 μ to 13.4 x 4.3 μ, mean spore size, with a grand average for the fifteen collections of 12.1 x 3.4 μ. The spores vary considerably in appearance. Mature material from Ohio is made up mainly of septate, cylindrical spores with homogeneous contents. Material from the west gave a larger percentage of immature and non-septate pycnospores (Pl. XXXVI, A, B, C, E). Co-type material collected by French closely resembled this western material.

PURE CULTURE STUDIES

All of the work was done with potato-1 per cent dextrose agar unless otherwise stated. Growth at room temperature.

Types on Vicia species. 1. *Vicia angustifolia* produces a white to buff fluffy growth very similar to *Ascochyta pisi* from pea (10), (13) with the production of carrot-red spore masses and the formation of pigment in the substratum (13).

2. *V. villosa.* Similar to above but some tendency to darker coloration in some isolations (New Jersey and Washington) approaching an olive color in some saltations. Rate of growth on various agar very close to *A. pisi* from pea (13). On agar long cylindric spores are produced, quite in contrast to the short cylindric ones on the host. (Compare Pl. XXXIV, E, F, H, J, K, M). Pigment occurs in substratum.

3. *V. faba.* Very similar to *A. pisi* on pea, less spore production, spores 14–21 x 4–6 μ, carrot-red in mass. Pigment in substratum.

Types on Lathyrus species. 1. *Lathyrus odoratus.* Material from Wisconsin, seed and pod. Rapid grower, slightly faster than *A. pinodella* (13). Forms a flat grey growth with frequent so-called saltations of lighter or sometimes darker strips and accompanied by the production of abundant small black pycnidia buried in the substratum, with only the upper half protruding. The substratum is made up of chlamydospores and dark compacted hyphae. The few tendrils of spores exuded are pale flesh to grey in mass and are made up chiefly of non-septate members. (To compare *A. pinodella* and *A. lathyri* as to spore characters (Pls. XXXV, I and XXXVI, F, G).

2. *L. tingitanus.* From foot-rot material sent by Dr. Weimer from Mississippi. Identical with *Mycosphaerella pinodes* (13).

Types on Medicago sativa. Isolations from various parts of the country, while varying greatly in morphology on the host, were indistinguishable in the test tube. Growth flat grey, felty with olive-brown patches. The felty growth is gradually superseded by a darker, flatter, carbonaceous growth in which numerous small black pycnidia are imbedded. The substratum consists of masses of chlamydospores and stromatic hyphae. The remains of the original felty growth, plus additional later tissue, obscures the pycnidia with a thin evanescent mycelial felt. Viewed from beneath, a plate culture presents an olive-brown appearance. Pycnidia exude pale pink spore masses made up largely of nonseptate spores. Rate of growth is rapid, paralleling that of *A. pinodella.*

On Melilotus stems, in culture, *A. imperfecta* produces only red-brown carbonaceous pycnidial masses that produce spores in quantities. Spore production may be obtained wholesale by use of water suspension inoculations on the surface of dextrose-potato agar or pea-soup agar.

INOCULATION STUDIES

In the work as many of the economic legumes grown in this country as time would permit were employed, including practically all those for food and forage, as well as a number of ornamental types.

The following types of Ascochyta were employed in inoculation work.

1. *A. pisi* from pea (6 isolations).
2. *M. pinodes* from pea (5 "). (One from *L. tingitanus.*)
3. *A. pinodella* (4 ").
4. *A. sp.* (*lathyri*) from *L. odoratus* (1, from Wis.)
5. *A. sp.* (*pisi*) " *V. villosa* (2 isolations).
6. *A. sp.* (*pisi*) " *V. faba* seed sent to the writer nine years after harvest in California (F. R. Jones). Fungus and seed both viable.
7. *A. imperfecta* from alfalfa (2 isolations).

Methods of inoculation of aerial parts. Plants were grown in four-inch pots in the greenhouse, from selected seed; and, when three weeks old, they were moistened under the tap and thoroughly sprayed with vigorous pycnospores in water suspension.

After inoculation the technique employed was similar to that described in previous work (13):

1. Incubation at 18–23° C. in spray chamber for 48–60 hours.
2. Use of check plants in all cases.
3. Plants removed from chamber and left in damp place for symptoms to develop; these were recorded daily.

TABLE 1.—*Results of inoculations on Vicia species (aerial). (Given in number of pots showing positive or negative.)*

	+1[1]	−	+2	−	+3	−	+4	−	+5	−	+6	−	+7	−
V. angustifolia	1	3	1	1	3				2	1		1		1
V. atropurpurea	1	2	2	1	3	1		1	2		1	2		
V. cracca		2	1		3	1		1	1	1	1			1
V. dasycarpa		3	1	1	3	3			1		1			1
V. ervilia	3	1	1	1	2			1	2		2	1		2
V. faba (2 types)	3	9	2	4	2	3		1	2		2	1		
V. monanthis	1	1	3	1	1		1	1	1		1			1
V. pannonica		6	2		2	2		1		2		1		3
V. villosa	5	8	2	3	3	4	1	1	5	1	1	5		2
V. sativa (2 types)	8	9	2	1	5	1	1		3	1	4	1		2
Total	22	44	17	13	27	15	3	7	19	6	13	12	0	13

[1] Number corresponds to number given source of culture at beginning of inoculation studies, for example, 1, *A. pisi* from pea, 2, *M. pinodes* from pea, etc.

4. Reisolations made and results recorded.

Methods of inoculation of subterranean parts. The various cultures were grown on sterilized peas in tubes and this material powdered and mixed with sterile soil in the same manner employed in previous work (13).

Results of Aerial inoculations. Out of some nine hundred odd pots of inoculated legumes, comprising sixty species and varieties of hosts, there were many that proved negative to attack from all Ascochyta species. Important among these were the following: five varieties of garden beans (*Phaseolus vulgaris*), soy bean, hyacinth bean, rice bean, and white and crimson clover.

On Vicia species. The results are given in table 1.

From the results obtained it indicated that most of the vetches are resistent to *A. pisi* from pea and are considerably more susceptible to Ascochyta isolated from vetches. A comparison with a summary of results on six varieties of peas shows this definite physiological trend. The figures 1 to 7 again refer to source of inoculum.

	1		2		3		4		5		6		7	
	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Number of pots.....	26	0	23	0	20	0	3	4	2	14	2	14	3	12

Inoculation studies brought out the fact that the blackish border on spots on *V. faba* was not due to fungal characters differing from those on other vetches but was due to a tendency of this particular host to blacken on injury. It may be compared to the habit of *Baptisia bracteata* (Muhl.) Ell. blackening on curing. Subtantiation of these statements was shown by the fact that Ascochyta isolated from *V. faba* developed light-centered red-bordered spots on other vetches.

In the vetch inoculations the spores and pycnidia retained their general characteristics through the several cycles of isolations and inoculations attempted. For example, the mean spore size of the large-spore Ascochyta from *V. faba* continued to be over 18 x 5 μ and the others somewhat smaller.

On Lathyrus species. The results are given in table 2.

Symptoms of inoculations from Ascochyta on *Lathyrus odoratus* are variable. Sometimes it causes dark brown lesions and leaf spots similar to those of *A. pinodella* and again, on the sweet pea, infection results in indefinite leaf and stem-tip injury similar to that given in literature for *A. lathyri* on various hosts. This condition is possibly due to rapid spread of the fungus in the succulent tip of the plant.

On Medicago, Trifolium, and Melilotus species. Alfalfa and bur clover (*Medicago hispida*) were immune from everything except *A. imperfecta* which was 100 per cent effective on them.

Melilotus indica, M. officinalis, T. hybridum, and *T. pratense* were negative to everything except about 20 per cent of the cases where inoculated

TABLE 2.—*Result of aerial inoculation of Lathyrus species. (Results given in number of pots positive or negative.)*

	+1[4]−		+2−		+3−		+4−		+5−		+6−		+7−	
L. clymaceum	2				1		1		1			2		2
L. latifolius		2		1	1	1	3	1		1				
L. odoratus	3	7		5	4	3	8	1	1	1	2	2		4
L. sativus	1		2		3		2	1	1		1	1		1
L. tingitanus		5	4		2		2	1	1	1		3		3
Total	6	14	6	6	11	4	16	4	4	3	3	8		10

[4] Numbers 1 to 7 refer to source of inoculum, see first paragraph under Inoculation Studies.

with *A. imperfecta*. The injury was in the form of a leaf scorching and rarely could be determined as Ascochyta injury except through reisolation.

On Phaseolus species. *P. aureus* and *P. aconitifolius* were susceptible in part to all pea-borne Ascochyta, but *P. mungo* and *P. multiflorus* were negative except for a small amount of *A. pinodella* in one inoculation. *P. lunatus* showed doubtful infection here, also. *P. aconitifolius* showed one case of infection with *A. imperfecta*.

On Miscellaneous hosts. Among various susceptible hosts not previously mentioned *Vigna sinensis, Cicer arietinum,* and *Lupinus polyphyllus* showed some susceptibility to *Mycosphaerella pinodes* and *Ascochyta pinodella.*

Length of period of incubation under conditions of inoculations.

Fungus	Host	Length of time required
Ascochyta pisi	pea	5–7 days
" (*pisi*)	*Vicia villosa*	do
" (*pisi*)	*V. faba*	do
" *pinodella*	pea	2½–3½ days
" (*lathyri*)	*L. odoratus*	3 –5 days
" *imperfecta*	*Medicago sativa*	3 –5 days
M. pinodes	pea	2½–3½ days

Results of subterranean inoculations. The results of some four hundred pots of plants were very inconclusive in the main. It was found, however, that *M. pinodes* could cause severe injury to *Cicer arietinum* and slight to moderate damage to vetches and Lathyrus species. *A. pinodella* was able to injure some of these types also, especially *Lathyrus tingitanus.*

A. pisi from pea and *Ascochyta* (*pisi*) from *Vicia villosa* seemed to be largely negative although there was some indication of a slight amount of a pale cortical rot.

ATTEMPTS TO DEVELOP ASCIGEROUS STAGES

Methods. Use of Melilotus stems and oat agar. Inoculation work done with potted plants. The plants were left for varying periods under green-

house and overwintering conditions. All of the pure cultures in stock were tried.

Results—Negative on all cultures of *Ascochyta pisi* from pea, Ascochytae from all vetches, *A. pinodella* from pea, *Ascochyta* (*lathyri*) from *Lathyrus odoratus* and *A. imperfecta* from alfalfa.

Perithecia answering to those of *Mycosphaerella pinodes* were obtained from such material isolated originally from pea, *Lathyrus tingitanus,* and *L. odoratus.*

DISCUSSION, OBSERVATIONS, AND CONCLUSIONS ON ASCOCHYTA ON VICIA SPECIES

The writer, being unable to obtain material of Stone's Mycosphaerella on vetches, has never observed any Ascochyta on vetches that does not fall close to *Ascochyta pisi* Lib. The symptomatology, absence of a perfect stage, cultural characters on media, and taxonomic traits seem to place the vetch forms in this country with *A. pisi.*

The extremely large spores of *A. pisi* on *V. faba* (Pl. XXXIV, A, B, C), are, together with their pycnidia, somewhat distinct from *A. pisi* as it occurs on the pea. Saccardo and Marchal (21) gives *A. pisi* f. *foliicola* with spores 15–18 x 4–5 μ on peas. Saccardo later (18, p. 303) thought this related to *A. boltshauseri* Sacc., a form usually found on Phaseolus spp. in Europe. Study of the California *V. faba* material and the brown, distinctly zonate spot caused by *A. boltshauseri* shows nothing in common (Pl. XXXVI, P, for spore characters of *A. boltshauseri*). Considering the great resistance of beans (*Phaseolus vulgaris*) to all types of *A. pisi,* we are safe in eliminating *A. boltshauseri.*

Various large-spore Aschochytae are listed on vetches. Jaap (8) mentions *A. viciae* on *V. sepium* with spores 18 x 5 μ. *A. orobi* Sacc. occurs on vetches as well as Lathyrus, according to European collectors. From German material seen and descriptions in literature the writer considers this species in part, at least, identical to *Ascochyta pisi* on vetches in this country.

Transitional types between those on the pea and the broad bean are common on *V. sativa* (Pl. XXXV, D, E, F), and on *V. angustifolia* Pl. XXXV, G) spores 15–25 μ in length are commonly found. Since it would be impossible to make more than highly artificial dividing lines between these types the writer considers that all forms on vetches with the typical symptoms of *A. pisi* on pea should be placed with this species.

Examination of type material sent through the courtesy of Drs. Sydow and Samuelsson of *A. ervicola* and *A. vicia-lathyroidis* leads the writer to place these under synonymy with the other types on vetches (Pl. XXXV, B, C, H). Peculiar slender-spore Ascochytae on vague, light tan spots on leaves of *V. caroliniana* and *V. americana* sent to the priter by Dr. J. J.

Davis do not seem to belong with the others and are left unclassified (Pl. XXXV, A).

The fruiting bodies of *A. pisi* on *V. faba* approach the pseudopycnidia of Potebnia (16), Diedicke (6), and others. Diedicke bases his genus Stagonosporopsis on the presence of loosely formed pycnidia, often acervulus-like, and the production of a variable percentage of multiseptate spores. An examination of many cultures and of host material and a study of *A.* (*Stagonosporopsis*) *boltshauseri* (5) (15) leads the writer to believe that the term pseudopycnidium is not valid for this particular group and that the genus Stagonosporopsis is untenable.

Technical description of Ascochyta pisi Lib. It seems advisable to give a revision or extension of the description of *Ascochyta pisi* to cover the range indicated in this paper. On account of small differences noted throughout the descriptions on various hosts, it will be necessary to divide this voluminous description into three parts for the sake of clearness and usefulness.

Ascochyta pisi Lib. on *Pisum sativum* L. and varieties. Spots definite, circular on leaves and pods, elongate on stems, yellowish brown with dark brown to reddish border which is often prominent, sometimes whitish in center, spots not defined on mature plant parts; pycnidia gregarious at center or in circular zones, somewhat prominent, depressed-globose, light golden brown to darker brown, ostiolate, 75–225 μ; spores hyaline with homogeneous contents and occasional oil drop, oblong, ends rounded, straight or somewhat curved, 1 (1–3) septate, somewhat constricted, 10–14 (6–19) x 3–5 (2.5–6) μ. Mean spore length usually less than 13.5 μ (12–15).

On leaves, stems, pods, and seeds of *Pisum sativum* L. and on aerial parts of *Lathyrus spp.* and *Vicia spp.* by artificial inoculations.

Ascochyta pisi Lib. on Vicia species. Spots definite, circular, occasionally elongate on leaves and pods, elongate on stems, at first yellowish brown to green, later white with dark red to brown border which is always prominent when mature, spots not defined on mature or senile parts; pycnidia gregarious at center or in circular zones, very prominent, depressed-globose, hyaline (light golden) to dark brown (pycnidia on stem parts often dark brown), ostiolate, 60–225 μ; spores hyaline, contents when mature homogeneous with rare oil drops, 1 (1–4) septate, cylindrical, sometimes curved, ends rounded, scarcely to somewhat constricted, 10–17 (8–25) x 2.5–5 (2–6) μ, mean spore length variable.

On leaves, stems, pods and seeds of *Vicia spp.* and by inoculation on aerial parts of *Lathyrus spp.* and slightly on *Pisum sativum.*

Ascochyta pisi Lib. on *Vicia faba* L. Spots usually definite, circular to elongate on leaves and pods, elongate on stems, at first dark brown or olive to black, later dirty yellow to white in center with dark brown to red border which is often prominent though sometimes diffused; pycnidia gregarious near center or in circular zones, very prominent, depressed globose, light brown, sometimes darker, parenchyma cells of walls usually not closely

packed, ostiolate, 80–250 μ; spores hyaline, contents homogeneous except frequent medium to large oil drops and crystalline-like material, 1 (1–4) septate cylindrical straight or somewhat curved, constricted at septum (sharply constricted in spores more than one-septate), ends rounded, 14–25 (12–28) x 3.5–6 (3–7) μ, mean spore length usually greater than 17 μ when mature.

On leaves, stems, pods and seeds of *Vicia faba* L. from Oregon and California and by artificial infection of other Viciae.

DISCUSSION, OBSERVATIONS, AND CONCLUSIONS ON ASCOCHYTA PINODELLA

From extensive collections recently sent to the writer by M. B. Linford and others the prevalence of *A. pinodella* in the United States is an established fact. It seems to be the commonest pisicolous Ascochyta in the arid regions of the West and it is found often in the East. Both this species and *A. pisi* were found associated with *Peronospora viciae* (Berk.) De Bary on pea leaves in a number of Linford's collections. The occurrence of Phoma or Phoma-like forms was commonly noted in some of Linford's material. Their nonpathogenicity was determined. Their presence may confuse.

The writer has found no collection of *A. pinodella* on anything except peas. This is surprising in view of the fact that some of the vetches are extremely susceptible to the organism under greenhouse conditions of inoculation. While hairy vetch and Hungarian vetch (*V. pannonica*) show a high degree of resistance, spring vetch (*V. sativa*) is nearly always killed to the ground. Pycnidia develop sparsely in some cases and with great profusion in others.

The very obscure nature of the common symptoms, those of a foot rot (in the main), no doubt allows many cases of this Ascochyta's depredations to pass unobserved.

DISCUSSION, OBSERVATIONS AND CONCLUSIONS ON MYCOSPHAERELLA PINODES

Through inoculation work it was shown that *M. pinodes* could attack a variety of hosts under the conditions of the experiment and often with great vigor. Its absence on vetches can be attributed, the writer believes, to a late-season parasitism that has not been observed by most workers who collected earlier in the summer. No doubt this is the explanation of Stone's material. It is known that rainy weather towards harvest may result in severe blighting of peas. Probably *M. pinodes* on pods of *L. odoratus* was a late September condition. Total negativeness of inoculation work on *L. odoratus* with cultures of *M. pinodes* bore this out.

The presence of *M. pinodes* in foot-rot material of *Lathyrus tingitanus* was clearly of a pathogenic nature.

DISCUSSION AND CONCLUSIONS ON ASCOCHYTA LATHYRI

Although similar to *Ascochyta pinodella* in several respects the small-spore Ascochyta on sweet pea in this country shows distinct spore characters, different period of incubation, different host range, and different cultural characters. In spite of lack of proof of connection with *Mycosphaerella ontarionensis* the writer considers the material to go under *Ascochyta lathyri*. (Pl. XXXV, K, L, M, N; Pl. XXXVI, F, G, for comparison of types.)

DISCUSSION, OBSERVATIONS AND CONCLUSIONS ON ASCOCHYTA IMPERFECTA

There can be little doubt that *A. imperfecta* is distinct from *A. medicaginis* as shown by inoculation work, coupled with study of material. Work with cultures of the latter as well as comparison with European types on alfalfa is necessary. (Pl. XXXVI, I, J.)

The surprising range of *A. imperfecta* in inoculation studies is not so remarkable when one considers that pycnidia and definite symptoms developed only on alfalfa and bur clover. The other hosts were plainly resistant and considering the young condition of the plants and ideal infective conditions it is rather doubtful if *A. imperfecta* would be found on these various hosts in the field. Bur clover no doubt may carry the trouble.

The variability of *A. imperfecta* in the field seems to be due to environment and not stable morphological differences. Under cultural conditions on media and through inoculation work as pointed out the various isolations were practically identical. (Compare Pl. XXXVI, A to E.)

The material collected by Simmons from Wyoming and Spokane, Washington, and referred to *A. pisi* var. *medicaginis* (Pl. XXXVI, H) by Saccardo is confusing. While spore characters are not unreconcilable with *A. imperfecta* the position of the pycnidia on the stem is unusual. Fresh material is needed before the position of this type can be made certain. The fact that the pycnidia on the stem of alfalfa are black and carbonaceous is due to their position and not to any morphological distinction. Extensive work with inoculations shows that, from a single inoculation, pycnidia may be obtained on the leaf that belong to Ascochyta and on the stem of the same plant that belong to Diedicke's genus Diplodina (6).

The writer would be inclined to place the material on alfalfa collected by Simmons under *A. imperfecta* with a question mark.

OBSERVATIONS ON ASCOCHYTA PHASEOLORUM SACC.

Two collections from Takoma Park, D. C., by A. Arnes on *Phaseolus lunatus* L. (*P. limensis*) show progressively greater increase in the percentage of septate spores (20 per cent) in August collection, 70 per cent, October) of material labelled *A. pisi*. The writer has compared this Takoma

Park material with foreign collections and finds it close to *A. phaseolorum*. The spores were short, cylindric, 7 x 2.5 μ mean spore size and the pycnidia were large, 135–180 μ. The spores were clearly septate and the cross walls were not to be confused with the false septations of the Phomopsis stage of *Diaporthe phaseolorum* (Pl. XXXVI, M to P).

Incidental work not to be presented here showed clearly that *Ascochyta phaseolorum* Sacc. and *Phyllosticta phaseolina* Sacc. were distinct.

SUMMARY

1. This paper is, in the main, a taxonomic study of Ascochytae on Leguminosae but treats of the life histories and physiological characteristics of the various forms and species as well as the mere observation of their morphological features.

2. Most of the North American collections on Viciae were shown to be similar or identical to *Ascochyta pisi* as it occurs on the pea. A form of *A. pisi* on the broad bean (*Vicia faba*) resembles *A. fabae*. Its difference in symptomatology from *A. pisi* on other vetches was shown to be due to host reaction. No ascigerous stage was found with any form of *A. pisi*.

3. Inoculation studies show *Mycosphaerella pinodes* to be pathogenic on a wide range of hosts, a fact not borne out by field and herbarium study. The writer has found this organism only on *Pisum spp., Lathyrus odoratus* and *L. tingitanus*. Stone's material on vetches was not examined. It is suggested that the occurrence of *M. pinodes* on vetches may be limited to late season.

4. A study of *A. pinodella* shows it to be common and widespread on the pea in America and under greenhouse conditions able to attack many hosts not found under field conditions as yet.

5. *Ascochyta lathyri* is the common species on *L. odoratus* in America.

6. *Ascochyta imperfecta* on alfalfa was shown to be distinct from *A. medicaginis* on *Medicago lupulina* and, while variable in the field, is clearly one species as far as leaf-spot symptoms go.

7. *A. phaseolorum* has been collected in America.

8. The genus Stagonosporopsis is invalid and Diplodina is of doubtful value.

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EXPLANATION OF PLATES

PLATE XXXIV

A. *Ascochyta pisi* on *Vicia faba*, leaf spot. Corvallis, Oregon.

B. *A. pisi* from *V. faba*, leaf spot caused by artificial inoculation with culture of pycnospores from California *V. faba* seed.

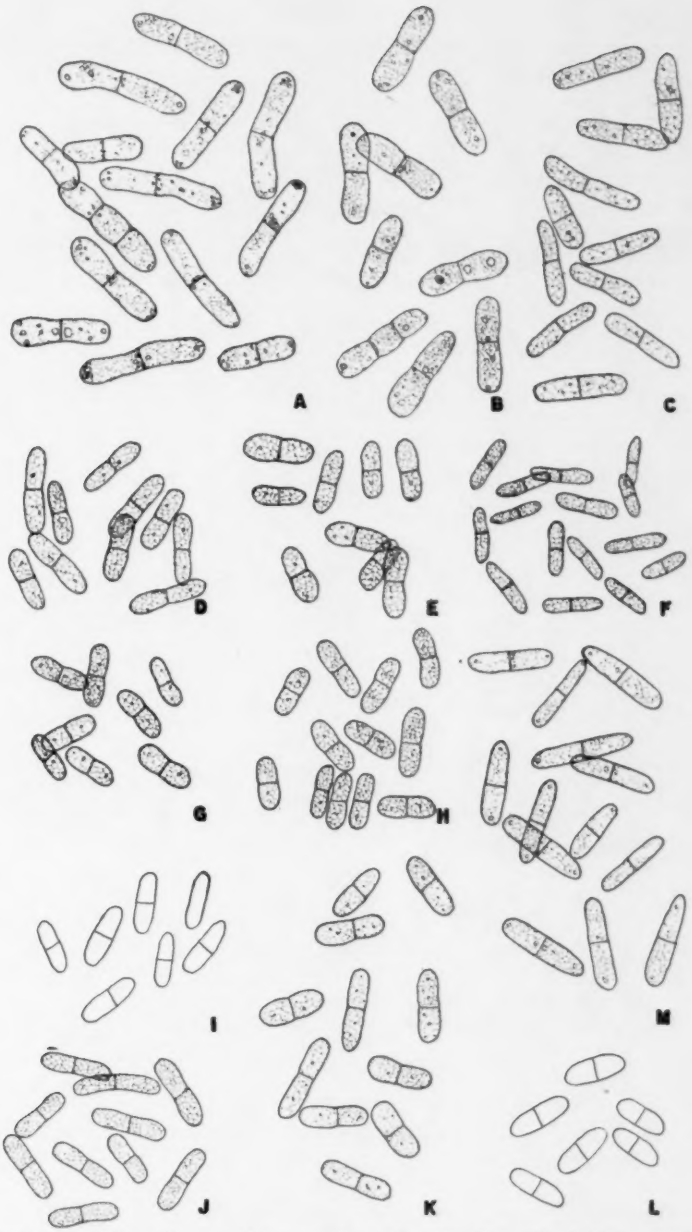
C. *A. pisi* from *V. faba* grown on potato-dextrose agar.
D. From artificially inoculated spot on *V. faba* with dextrose agar culture of *A. pisi* isolated from pen.
E. Same as D but inoculum obtained from *V. villosa*, Pullman, Wash.
F. From culture of *A. pisi*, isolated from pea, on potato-dextrose agar.
G. From artificially inoculated spot on *V. sativa* with *A. pisi* from pea.
H. *A. pisi* from leaf spot on *V. villosa* (Pullman, Wash.).
I. Same type as fig. H, from suecia, collected by Vestergrun.
J. On potato-dextrose agar, material from fig. H.
K. Same as J but source is Cincinnati, Ohio.
L. *A. pisi* on *V. villosa*, leaf spot. Valentine, Nebr., J. M. Bates. 1897.
M. On potato-dextrose agar, material from fig. H.

PLATE XXXV

A. Ascochyta species on *V. Americana* from Wisconsin (J. J. Davis).
B. Type material of *Ascochyta ervicola.*
C. Type material of *A. vicia-lathyroidis.*
D. *A. pisi* on *V. sativa.* Leaf spot, Mississippi.
E. Same as D from London, Ontario (Dearness).
F. Same as E from Russia (Myc. Rossica 37).
G. *A. pisi* on *V. angustifolia* from Wisconsin.
H. *A. pisi* on pod of *V. hirsuta* from Maine.
I. *A. lathyri* on *L. odoratus* from Minnesota.
J. *A. pisi* isolated from pea and inoculated onto *Lathyrus odoratus.*
K. Potato-dextrose agar culture of *A. lathyri* isolated from pod of *L. odoratus*, Wisconsin.
L. *A. pisi* on *Lathyrus vernus* Germany (Oertel).
M. *A. lathyri* on pod of *L. odoratus*, Wis.
N. *A. lathyri* on everlasting pea (?), New Jersey.

PLATE XXXVI

A. *Ascochyta imperfecta* from alfalfa. Leaf spot. Utah.
B. Same from Newtown, Ohio.
C. Same from Cincinnati, Ohio.
D. Isolation from Cincinnati, Ohio, *A. imperfecta* on potato-dextrose agar.
E. *A. imperfecta* on alfalfa. California material (Lee Bonar).
F. *A. pinodella* on pea. Leaf spot. Utah.
G. *A. pinodella*, leaf spot, Florida.
H. Ascochyta (*A. pisi* var. *medicaginis* Sacc.) on stems of alfala, Wendover, Wyoming. (Simmons.)
I. *A. medicaginis* on *Medicago lupulina.* Oxford, Ohio.
J. Same as fig. I. London, Ontario (Dearness.)
M. *A. phaseolorum* on *Phaseolus lunatus.* Takoma Park, D. C. Oct. 1908.
N. Same type as N. (Petrak Myc. Carpat. 402.)
O. Same as N. (Philippines, C. V. Piper.)
P. *A. boltshauseri* on *Phaseolus sp.* Albania (Petrak).



Pycnospore drawings from water mounts made with the aid of the camera lucida. ×1600. Spores with contents plasmolyzed through age or treatment are shown in outline only.

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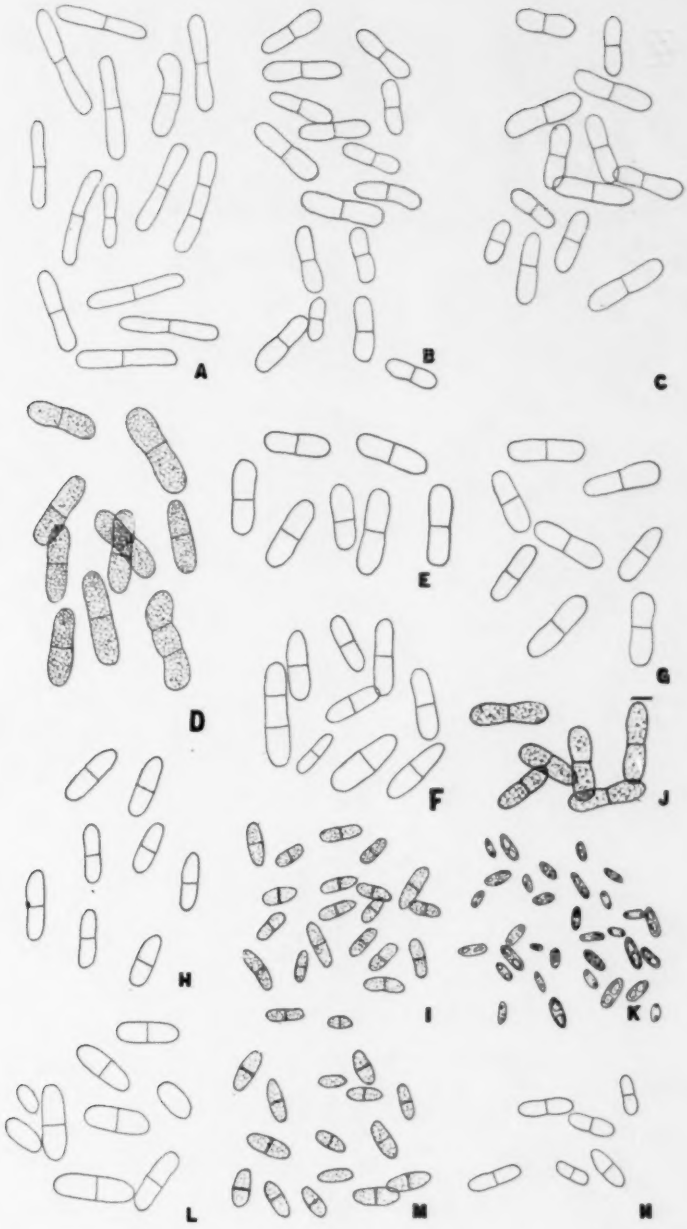
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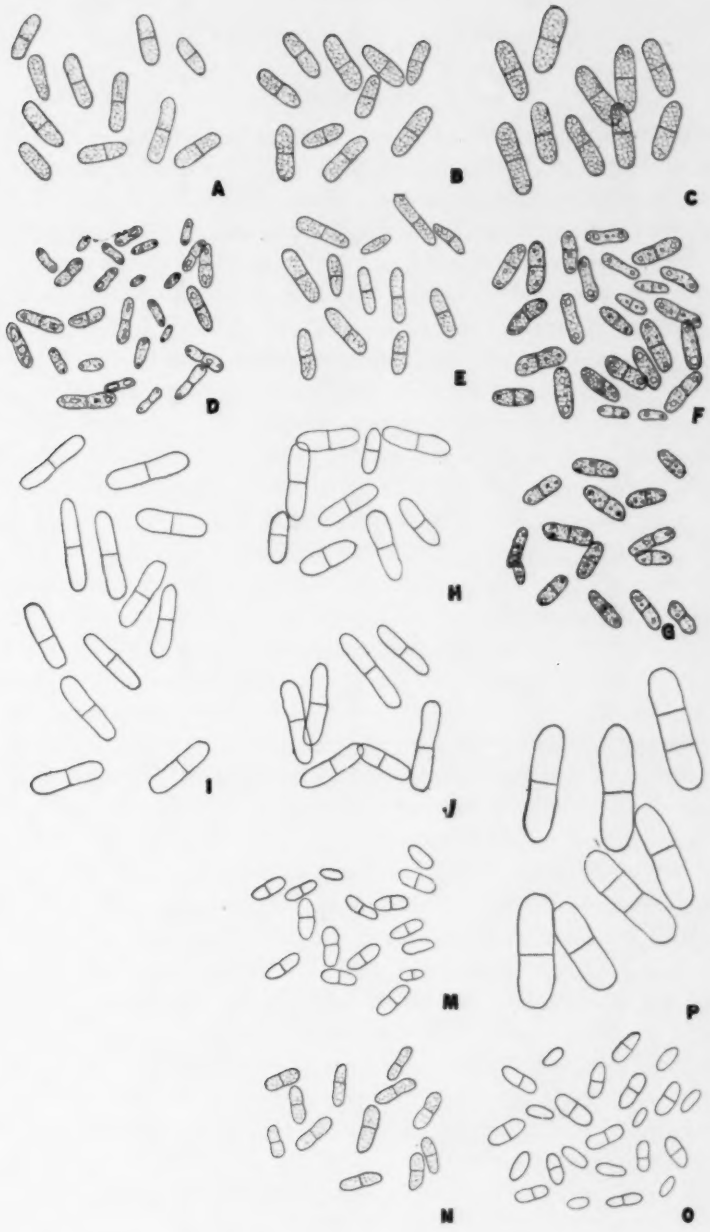
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MASKING OF SWEET-POTATO MOSAIC

L. L. Harter and W. A. Whitney

INTRODUCTION

A curious physiological phenomenon with respect to the mosaic of sweet potatoes (*Ipomoea batatas* Poir.) was accidentally discovered during the winter of 1926 and 1927 with plants growing in the greenhouse. In the fall of 1926 a number of roots from mosaic-infected Nancy Hall plants were bedded in a box which was located on a side bench above some steam pipes. The potatoes germinated normally and in about six weeks vines of considerable length had developed. The leaves were typically mosaic, being dark-green, deeply lobed, and distorted, and the surface covered with numerous white lines, closely resembling pencil marks, running indiscriminately in all directions. The foliage was so abnormal for the variety that one unfamiliar with it might easily have regarded it as a different variety.

It so happened that one of the vines accidentally grew over the side of the bench and hung in such a position that the steam, escaping in a considerable volume from a loose-fitting valve, not more than two feet distant, constantly flowed over it. While no records were made of the temperature, it was certainly very high, for it was uncomfortably warm in the vicinity of the vine. Exposed to high temperatures, the vine grew rapidly, and in a few days it was observed that the new leaves lacked some of the mosaic symptoms characteristic of the older leaves on the vine. The first leaves which unfolded under these conditions were not entirely normal when mature, neither were they typically mosaic. However, each succeeding leaf more closely approximated that of a healthy plant and more remotely that of the typical mosaic. During a period of about three weeks under these conditions the vine, about 2½ feet long, bore apparently normal leaves at the distal 8 inches. The next 8 inches bore leaves with symptoms varying from typical mosaic to nearly normal leaves and the remainder of the vine, as well as all the several other branches and shoots, was typically mosaic.

In order to determine if the virus[1] causing the mosaic was absent from the portion of the vine bearing normal leaves, it was severed on the distal side of the first normal leaf about 6 inches back of the growing point, in order to remove only healthy tissue, and planted in a pot of sandy loam soil. The pot was placed in another part of the greenhouse where the temperatures ranged from 21° to 27° C. The cutting rooted and developed new growth which was typically mosaic, showing that the virus was not destroyed but only temporarily inactivated.

[1] The term "virus" as used in this paper is employed to designate the yet unknown causal principle.

TEMPERATURE AND HUMIDITY RELATIONS

From the behavior of this plant it was thought that temperature and humidity might be the controlling factors in the masking of mosaic symptoms. To test the validity of these suppositions an ordinary infection chamber, constructed of glass and located in a greenhouse, was equipped with a thermostatically-controlled heating unit, wet- and dry-bulb thermometers (for the determination of relative humidity), a pan of water, and a thermograph. A mosaic-infected plant (Fig. 1) was then placed in the chamber. The temperature fluctuated from 30° to 41.1° C. but averaged

FIG. 1. A typically mosaic-infected plant used in one of the experiments. (Compare with Fig. 2.)

38.8° C., with an average relative humidity of 65.5 per cent. While sweet potatoes ordinarily will endure a high temperature, the leaves were unable during the winter to withstand the sudden shock of exposure to an extremely high temperature and gradually turned yellow and died. The roots and the older, hardier stems, survived, however, and after a period of acclimatization to the higher temperature, usually about four or five days, new branches and leaves developed. When the greenhouse became warmer with the approach of summer, the leaves did not die after being placed in the infection chamber to the same extent that they did during the winter and early spring. The new leaves appeared healthy in every respect. After the plant had developed a new growth of vines and leaves it was removed from the chamber and placed on a side bench where, in the course of a few weeks, other shoots (Fig. 2) developed, bearing leaves identical with those present when the plant was first placed in the chamber.

In these preliminary experiments an attempt was made to simulate the conditions that obtained when the steam flowed over the pendant branch. In order to study the effect of humidity and temperature on the masking of mosaic in more detail and under relatively-controlled conditions, two additional infection chambers were constructed. The chamber previously used, hereinafter designated as number one, was maintained as formerly; chamber two was equipped with thermostatic heat control alone; and chamber three was provided only with a pan of water to maintain a high relative humidity. Wet- and dry-bulb thermometers and a thermograph were placed in each chamber. Thus, in the chambers the effect of temperature and humidity, each acting alone and together, could be studied. Typical mosaic plants were selected, photographed, and one placed in each chamber. Readings of the wet and dry bulbs were made daily and water was added to the pots in sufficient amount to insure a normal growth.

The plant supplied with additional heat alone developed new shoots in about 4 or 5 days, and on the seventh day it was evident that the new foliage was typical of normal plants. The plant subjected to both additional heat and increased humidity developed new healthy shoots a few days later. Luxuriant growth was made by the plant in chamber 3, where the greenhouse temperature prevailed, and only the humidity was increased. The foliage of the third plant, while approximating the normal in appearance, differed from it in that the lobes were slightly deeper, somewhat malformed, and the veins lighter in color. In table 1 is summarized the temperature and humidity records taken during this experiment.

The plants were removed from the chambers at the end of 33 days and photographed, and then placed on a bench in the greenhouse where the tmperature was lower. In about a week the new growth showed typical symptoms of mosaic.

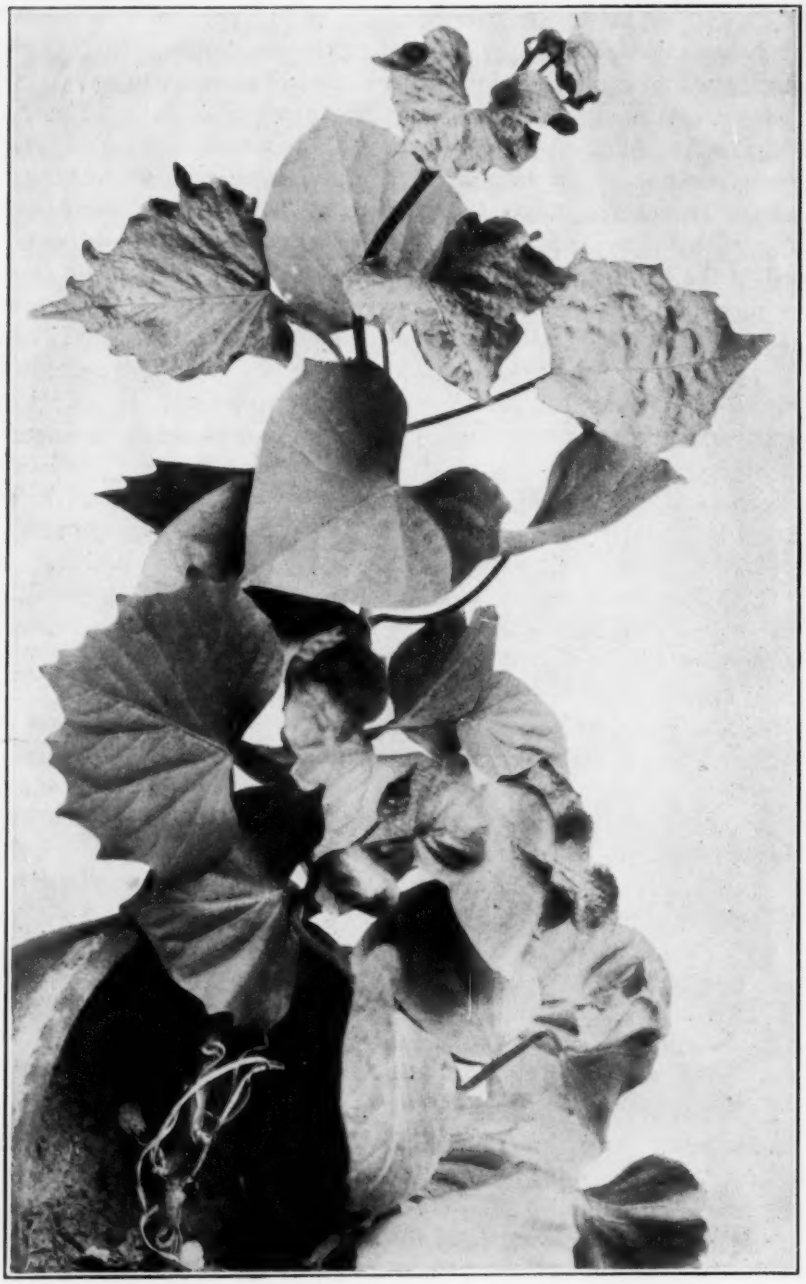

FIG. 2. A plant eleven days after removal from the chamber (37.8° C.). Note the typical mosaic symptoms on the new growth.

TABLE 1.—*Temperature and humidity in chambers 1 to 3*

Chamber No.	Treatment of Chamber	Temperature in °C.		Relative Humidity—Percentage	
		Range	Average	Range	Average
1	Temperature and humidity raised	35.0–42.7	40.8	46.0–95.9	72.5
2	Temperature only raised	33.3–45.6	38.3	44.0–85.0	59.3
3	Humidity only raised	22.8–40.0	32.8	79.5–100.0	92.0

These experiments were repeated several times with identical results.

EFFECT OF SOIL TEMPERATURE

In the above experiments the roots and aerial parts of the plant were subjected to the same temperature. There was, therefore, no evidence to show whether the change in the appearance of the foliage was due to the influence of the high temperature on the aerial parts of the plant alone, on the roots alone, or on both together.

Six constant-temperature tanks in a greenhouse were equipped with thermostatic heat control, and two each held at average temperatures of 39.8, 29.8, and 19.8° C. Each tank accommodated 8 pots which projected into the water 8 inches, thus subjecting 16 pots to each temperature. The plants were carefully selected and arranged so that there were 12 mosaic-infected and 4 normal ones at each temperature. The aerial parts of the plant were exposed to the greenhouse temperature which could not be controlled. The experiment, which lasted 31 days, was conducted in October and part of November at a time when the temperature of the greenhouse was not high at any time. A good growth was made at the two highest temperatures (39.8, 29.8° C.) and but little growth at the other (19.8° C.). Previous experiments showed that when the entire plant was subjected to a temperature approximately 40° C. mosaic was masked, the plant producing only normal-appearing foliage. The results of these experiments, however, show that a similar temperature about the roots had no effect on the foliage whatsoever, the new growth being in every instance typically mosaic.

AIR AND SOIL TEMPERATURE IN COMBINATION

Inasmuch as mosaic was not influenced by the temperature of the soil, the effect of various combinations of soil and air temperatures was studied, using two of the constant-temperature soil tanks described above. At the

outset one of these tanks (No. 1) was set to run at about 40° C. and the other (No. 2) at approximately 26° C. A glass chamber was placed over the front half of Tank No. 1 and the temperature therein adjusted to about 40° C. In each half of the tank was placed three mosaic-infected plants and one healthy one. Thus the four plants in the chamber were exposed to a soil and air temperature of 40° C. and the other four plants exposed to a soil temperature of 40° C. and to the air temperature of the greenhouse (about 23° C.). In Tank No. 2 the soil temperature was adjusted to about 26° C., a chamber placed over one half and a series of plants similar to that of Tank No. 1 set therein. In this tank half of the plants were subjected to a soil temperature of 26° C. and an air temperature of 40° C., the other half of the plants being exposed to a soil temperature of 26° C., with the air temperature that of the greenhouse.

A combination of air and soil temperature of 40° C. was too high, the plants dying in a few days. For this reason the water in Tank No. 1 and the air temperatures of the chambers over the two tanks were lowered to about 35° C. Plants exposed to these temperatures thrived both inside and outside of the chambers. The mosaic-infected plants within the chambers developed healthy-appearing foliage in about 10 days, while those outside continued to produce leaves infected with mosaic. The controls remained healthy in all cases. The experiment was repeated, the water in Tanks No. 1 and No. 2 being maintained near 35° and 25° C., respectively, and the chambers at an average temperature of 35° C. The experiment was terminated at the end of 45 days, with the result that none of the mosaic plants outside of the chambers showed any healthy growth, while normal foliage was produced by mosaic plants within the chambers, whether the soil temperature was 35° C. or 25° C.

DISCUSSION OF ENVIRONMENTAL RELATIONS AND MASKING

This reduction of mosaic symptoms may properly be classed as masking and, although rather well known for other mosaic diseases, it is the first time it has been reported for sweet-potato mosaic. It must not be overlooked that, due to our limited knowledge of the causes of mosaic diseases in general, no satisfactory explanation can be offered for this interesting phenomenon.

One of the earliest references to masking—then not so called—due to environmental conditions is that of Sturgis (8), who in 1899 observed that tobacco grown under shade is less severely attacked by mosaic than when grown in the open. Johnson (4), working with the same crop, showed that under controlled temperature conditions, the mosaic symptoms decreased above 30° C. and disappeared at 36–37° C. He (5) further showed that soy-bean mosaic was inhibited by temperatures of 26–28° C., slightly

higher than those for potatoes; that mosaic of pea beans resist higher temperatures; and that the mosaic diseases of clover and tomatoes (resulting from inoculation with tobacco mosaic) were inhibited by temperatures similar to those which masked mosaic of tobacco. Field observations of aster yellows and experimental work with mosaic of cucumbers led Doolittle (2) to conclude that environmental conditions affect the seasonal severity of these diseases, the degree being correlated with the vigor of the plants—the more vigorous the plant the shorter the mosaic incubation period. Elmer (3) came to the same conclusions, and stated that the appearance of mosaic is correlated with the vitality of the plant and not with any attenuation of the virulence of the virus involved. Tompkins (9) working with mosaic of potatoes concluded that air temperature is the only factor effective in masking and that only relatively short exposures at the critical temperatures—23–24° C.—are needed to mask the symptoms and also that the rate of masking was dependent on the length of exposure and the age of the leaves. On removal to a lower temperature the symptoms returned. Histological study showed that there was a difference between masked and normal leaves. Wilcox (10) observed that certain types of raspberry mosaic are masked during hot weather. Leaves that were mottled during cool weather remained so but the new growth was healthy.

That certain plants are carriers of mosaic is a theory advanced to explain the occurrence of mosaic diseases in crops where there is no apparent source of inoculum. *Physalis alkekengi* L. was shown by Nishimura (7) to carry the mosaic disease of tobacco, although itself exhibiting no external symptoms. *Nicotiana glauca* Graham may, according to Allard (1), show but slight symptoms of mosaic, yet the expressed juices be highly infectious to tobacco. Melhus (6) found that *Physalis longifolia* Nutt. carried mosaic over the winter and transmitted it to various solanaceous plants. He also showed that mosaic symptoms are masked on eggplants that have passed beyond the seedling stage.

The suppression of mosaic symptoms in the Nancy Hall sweet potato occurs when the plants are exposed to high temperatures whether or not in the presence of a high relative humidity. The most rapid and complete masking occurred in chambers with relatively low humidity and high temperature. In chambers with high temperature and high humidity masking took place more slowly. A partial masking, whose degree increased with an increase in temperature, occurred in the chambers with a variable temperature and high humidity. During the first part of one experiment, there was a period when the greenhouse was quite cool, especially during the night, and the temperature of the chamber with no added heat dropped quite low. Under these conditions practically no masking occurred, but with the resumption of warmer weather and the consequent rise in the

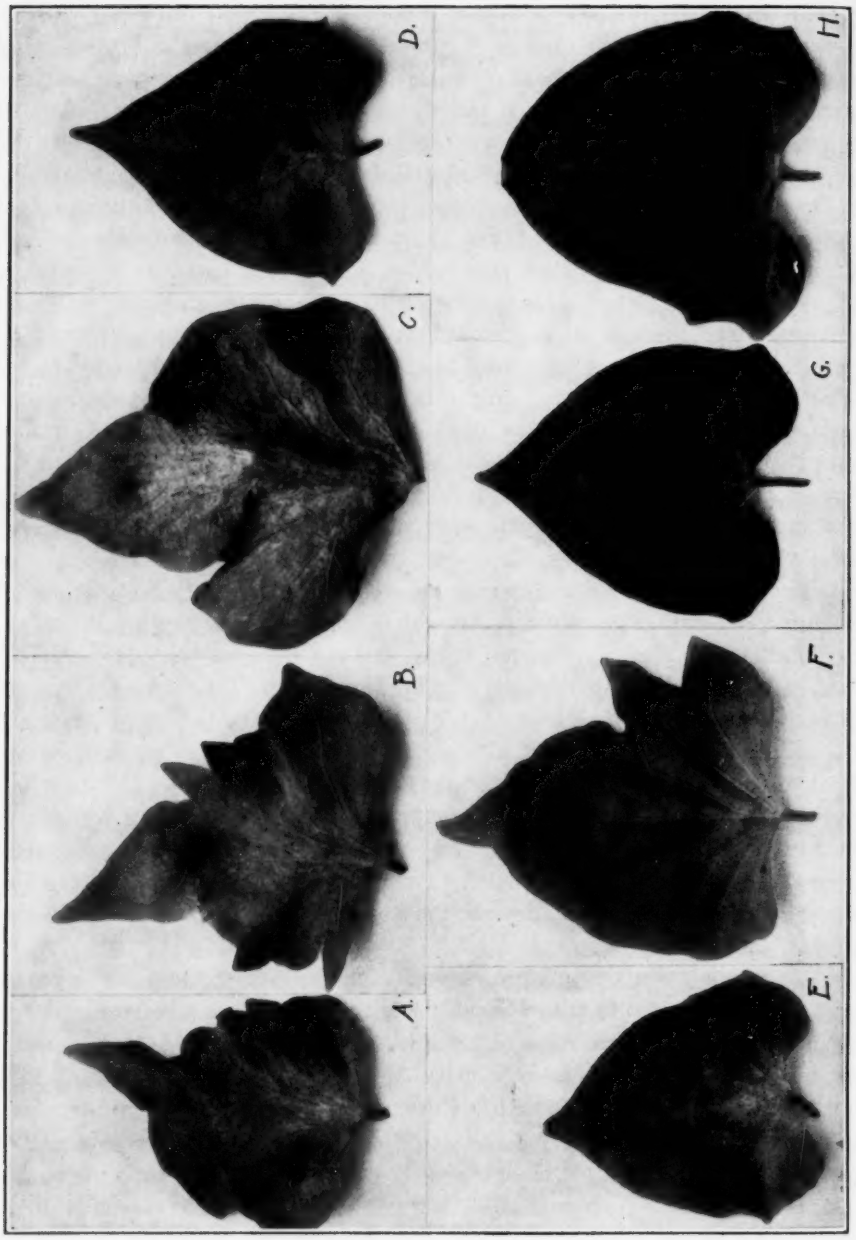

FIG. 3. A series of leaves showing the transition stages from mosaic to healthy leaves under the influence of an air and soil temperature of about 35° C. and an average air relative humidity of 87.5 per cent.

temperature of the chamber masking became apparent, although the degree was markedly less than in any one of the other chambers.

The first new leaves which develop under the influence of a high temperature are not fully normal, but each succeeding leaf more nearly approximates the healthy ones. Figure 3 shows a series of eight leaves and illustrates the various steps in the change from mosaic to healthy leaves. These leaves were grown under the influence of high temperature and taken in sequence from a single vine, with the exception of H which is from a healthy plant. An inspection of the illustrations shows considerable malformation in leaves A–C but with a tendency to normal shape and size. Leaves D and E are normal in all respects, except that there still remains some of the white marking at the base of the leaf. Leaf F is lobed on one side, while leaves G and H are both normal for the variety, leaf H having been healthy from the beginning. Mosaic leaves never change to normal leaves. Normal leaves on a mosaic plant never become mosaic, regardless of the conditions under which they are held.

It appears, as was found by Tompkins (9) with regard to potato mosaic, that temperature is the primary factor governing masking, with humidity playing a slight counteracting rôle. The exact range of temperatures at which masking takes place is not definitely known. It is safe to assume, however, that sweet potato mosaic falls into the high temperature group along with that of tobacco (4) and of clover (5).

SUMMARY

Masking of the mosaic symptoms of sweet potatoes (*Ipomoea batatas*) as a result of high temperature is reported.

The symptoms in the new growth are completely masked at a temperature of about 38° C. with or without an accompanying high humidity. A comparatively high relative humidity seems to retard rather than augment masking.

Mosaic symptoms recur on the new growth following the removal of the plants to lower temperatures. All changes take place on new growth alone; old growth, whether healthy or diseased, does not alter.

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THE EFFECT OF BORDEAUX MIXTURE ON THE INTERNAL TEMPERATURE OF POTATO LEAFLETS[1]

PAUL E. TILFORD AND CURTIS MAY

Several factors have been suggested as the cause of the physiological effect of Bordeaux mixture on potato plants. Among these are a stimulation by some of the ingredients of the spray, the alteration of the transpiration rate, an increase in the assimilatory capacity of the leaves, and the shading of the leaf by the Bordeaux film. No attempt will be made here to review this literature except in so far as it bears directly on the present problem.

Schander (13) measured the temperature of sprayed and unsprayed leaves of *Pachysphytum bracteosum* by placing a thermometer back of leaves exposed to the sun. He obtained no consistent differences. Lutman (10) concluded that potato leaves sprayed with Bordeaux mixture must be cooler than unsprayed leaves, since a thermometer held under a glass slide sprayed with Bordeaux mixture registered one-third to two-thirds of a degree Centigrade lower temperature than when exposed to direct sunlight.

According to Butler (2), the physiologic response resulting from Bordeaux mixture on potato plants, depends on the intensity of the shadow cast by the Bordeaux film.

The writers have been unable to find in the literature, thoroughly reviewed by Clum (3) (4), Ehlers (8), and also by Miller and Sanders (12), any accurate data on the influence of Bordeaux mixture on leaf temperature.

METHODS

The method of recording the leaf temperatures was essentially the same as that employed by Clum (3). Copper and Constantin thermocouples made of very small wire and equipped with a ten-foot extension were used.[2] A Leeds and Northrup type K potentiometer, type R galvanometer, Eppley standard cell, storage batteries, and a thermos bottle constituted the equipment. The thermocouples were calibrated by making voltage readings over a series of known temperatures. One junction was kept at a constant low temperature by inserting it in a thermos bottle filled with chipped ice and distilled water. The temperature of this cold bath was continually checked with a one-tenth-degree thermometer. The other junction was

[1] Published with the permission of the Director of the Ohio Agricultural Experiment Station.

[2] The thermocouples were purchased from the Cambride and Paul Instrument Company, Grand Central Terminal, New York, N. Y.

placed in water baths of known temperature. In making the readings the tip of the thermocouple was held as closely as possible to the bulb of a tenth-degree-thermometer and at the same instant that the voltage was read the thermometer reading also was noted. By plotting the voltages as abscissas and temperatures as ordinates a straight line was secured from which voltage readings could readily be converted to temperature readings. The wires directly back of the tip of the couple were reinforced with De Kotinsky cement. This made it possible to insert the point into a leaf without danger of breaking the fine wires.

The instruments were set on a solid table in the greenhouse and protected from the sun by heavy burlap curtains. Uniform potato plants, 15–18 inches high, were used. The upper and lower surfaces of the leaves of two plants were sprayed with one material and of two similar plants with another. They were well watered an hour before readings were made so the leaves would be turgid. When readings were to be made one unsprayed and one sprayed plant, or two plants sprayed with different materials, were set side by side on a box in full sunlight. The thermocouple was inserted into the lower side of the midrib of a leaflet which was then held about perpendicular to the sun's rays. As soon as the voltage became stable the reading was made. The thermocouple was then inserted into a leaflet of the second plant and a reading made. It was found that readings could not be made in less than one to one-and-a-half minutes because, immediately after inserting the thermocouple, the voltage was too high. In the course of a minute to a minute-and-a-half the voltage dropped to a lower and stable point. This effect was due probably to the wounding of the tissue when the couple was inserted.

The couple was inserted in the midrib because the mesophyll of the lamina of potato leaflets is so thin that the couple could not be placed between the upper and the lower epidermis. Smith (15), however, has shown that readings taken in the midrib are comparable with those taken in the lamina of leaves.

Ten readings were made on each plant. The first pair of plants was then replaced by a second pair and a similar set of readings taken, making twenty determinations for each comparison. Sometimes, but not often, more than one or two readings were made on a single leaflet. All the determinations were made during February and March in the greenhouse.

RESULTS

Results were obtained comparing the temperature of leaves of plants sprayed with 4–4–50 Bordeaux and unsprayed leaves during both cloudy and sunny weather; 4–4–50 and 4–6–50 (hydrated lime) and 4–4–50 and 20–80 copper lime dust. These data are given in table 1.

TABLE 1.—*Comparison of the cooling effect of different Bordeaux mixtures and copper-lime dust on the internal temperature of potato leaves*

Shade—Cloudy weather			Sun shining brightly								
4–4–50	No spray	Diff. in favor of 4–4–50	4–4–50	No spray	Diff. in favor of 4–4–50	4–6–50	4–4–50	Diff. in favor of 4–6–50	4–4–50	20–80 dust	Diff. in favor of 4–4–50
°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
20.0	20.1	−0.1	25.6	27.7	−2.1	26.1	25.8	+0.3	30.1	29.3	+0.8
20.6	20.6	0.0	26.6	27.9	−1.3	26.4	32.9	−6.5	29.6	28.7	+0.9
20.6	21.3	−0.7	23.9	25.6	−1.7	26.4	26.1	+0.3	29.5	31.2	−1.7
21.2	21.8	−0.6	24.3	26.3	−2.0	27.7	28.2	−0.5	26.3	27.5	−1.2
20.8	21.1	−0.3	24.0	25.0	−1.0	24.3	27.1	−2.8	28.5	28.2	+0.3
19.2	19.2	0.0	24.5	24.8	−0.3	26.3	25.7	+0.6	28.6	27.8	+0.8
18.9	18.9	0.0	24.9	24.7	+0.2	27.3	27.0	+0.3	27.9	28.2	−0.3
19.2	18.9	+0.3	24.5	25.7	−1.2	26.2	27.9	−1.7	27.6	26.8	+0.8
19.5	18.7	+0.8	25.8	24.5	+1.3	26.5	26.2	+0.3	29.3	28.0	+1.3
19.5	20.0	−0.5	21.3	24.3	−3.0	27.0	29.5	−2.5	29.2	27.8	+1.4
19.8	19.9	−0.1	23.0	25.6	−2.6	30.5	30.4	+0.1	26.1	27.9	−1.8
22.1	23.7	−1.6	23.7	25.6	−1.9	28.8	28.3	+0.5	25.2	26.9	−1.7
22.4	23.7	−1.3	29.1	29.1	0.0	28.5	28.9	−0.4	25.5	25.8	−0.3
22.0	23.1	−1.0	25.9	30.0	−4.1	28.7	29.4	−0.7	24.6	26.5	+0.1
22.7	21.1	+1.6	25.4	26.0	−0.6	29.7	31.1	−1.4	24.9	26.5	−1.6
22.9	22.9	0.0	26.4	28.5	−2.1	29.3	29.6	−0.3	24.9	26.1	−1.2
23.1	22.2	+0.9	27.8	29.3	−1.5	27.8	30.1	−2.3	26.3	26.4	−0.1
22.8	22.0	+0.8	28.0	29.0	−1.0	29.7	30.0	−0.3	25.3	25.6	−0.3
23.7	24.2	−0.5	26.6	27.9	−1.3	29.8	30.2	−0.4	26.6	25.9	+0.7
23.7	22.5	+1.2	24.8	27.7	−2.9	29.0	30.9	−1.9	25.6	25.6	0.0
Average difference		−0.055			−1.45			−0.96			−0.15
Odds		1:1		Greater than	10000:1			113:1			2.6:1

Leaves sprayed with Bordeaux plus 1 per cent lamp black were compared with unsprayed leaves; in addition, 4–4–50 Bordeaux was compared with a lime spray (6 lb. to 50 gal. water). Finally, measurements also were made on the leaves of unsprayed plants to determine what variability might be expected. These data are given in table 2.

TABLE 2.—*Comparison of the internal leaf temperatures of potato plants treated in different ways*

No. 1 No spray	No. 2 No spray	Diff. in favor of No. 2	No spray	4–4–50 plus 1 per cent lamp black	Diff. in favor of no spray	0–6–50 lime	4–4–50	Diff. in favor of lime
°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
27.4	27.7	+ 0.3	27.1	34.3	– 7.2	24.3	24.1	+ 0.2
27.8	27.2	– 0.6	32.0	37.4	– 5.4	24.7	25.4	– 0.7
27.2	28.4	+ 1.2	29.7	34.6	– 4.9	26.4	26.8	– 0.4
28.7	27.9	– 0.8	31.7	32.7	– 1.0	26.3	28.3	– 2.0
30.6	29.8	– 0.8	29.9	34.2	– 4.3	26.8	27.1	– 0.3
30.9	33.4	+ 2.5	28.8	33.0	– 4.2	28.2	28.0	+ 0.2
34.3	32.8	– 1.5	27.5	30.7	– 3.2	26.9	28.3	– 1.4
32.9	31.6	– 1.3	27.3	29.2	– 1.9	26.0	27.2	– 1.2
31.1	31.1	0.0	28.4	33.4	– 5.0	27.0	28.6	– 1.6
30.9	31.4	+ 0.5	31.3	36.9	– 5.6	27.9	28.8	– 0.9
31.9	32.4	+ 0.5	32.7	33.2	– 0.5	25.9	28.0	– 2.1
31.8	32.4	+ 0.6	33.2	34.2	– 1.0	28.9	27.0	+ 1.9
32.2	31.6	– 0.6	31.1	33.3	– 2.2	27.8	29.8	– 2.0
33.9	32.2	– 1.7	31.7	34.6	– 2.9	29.7	31.2	– 1.5
34.4	36.3	+ 1.9	31.7	33.0	– 1.3	28.8	28.8	0.0
33.1	33.3	+ 0.2	31.0	33.0	– 2.0	26.8	27.8	– 1.0
33.0	33.0	0.0	32.1	34.1	– 2.0	28.2	30.9	– 2.7
30.7	31.4	+ 0.7	32.0	33.0	– 1.0	28.2	28.7	– 0.5
30.6	31.3	+ 0.7	30.3	32.0	– 1.7	28.9	30.9	– 2.0
33.8	31.4	– 2.4	30.7	31.9	– 1.2	27.4	28.0	– 0.6
Av. Difference		– 0.025			– 2.92			– 0.93
Odds		1.18: 1	Greater than		10000: 1			4999: 1

At first sight the readings may appear more variable than one would expect. However, when the factors causing this variability are understood, the results are as consistent as could be hoped for. Changes in light intensity, air-convection currents, the angle of incidence of the sun's rays, the age of the leaf, length of exposure to direct sun rays, and the state of turgidity of the leaves have been pointed out by Eaton and Belden (7) as factors influencing rapid changes and variations in leaf temperatures. Variations in the thickness of the spray coating undoubtedly account for

some of the variations obtained in the results here reported. While every possible precaution was taken to minimize these factors or, at least, to have them comparable throughout a series of readings, yet slight variations occurred. Clum (4) reports similar experiences. When he placed thermocouples in the right and left sides of a cabbage leaf, and then placed the leaf so that both sides were exposed to the sun approximately alike, he found that in two-thirds of the readings the temperatures on opposite sides of the leaf varied about one degree Centigrade. Consequently, several readings are necessary to obtain significant results.

It was possible to calculate "t" for the mean in each case, since the readings were taken in pairs. The odds that the results are significant were obtained from the revised tables of "t" by Student (16). It is clearly evident from the data presented that 4–4–50 Bordeaux mixture has a cooling effect on leaves when exposed to the sun. This is not true, however, in cloudy weather. Leaves sprayed with 4–6–50 were significantly cooler than those sprayed with 4–4–50 Bordeaux. Also, leaves sprayed with lime suspension, 6 pounds to 50 gallons, were cooler than leaves coated with 4–4–50 Bordeaux. Copper-lime dust produced the same cooling effect as 4–4–50 Bordeaux. Leaves sprayed with Bordeaux plus one 1-per-cent lamp black were decidedly hotter than unsprayed leaves.

DISCUSSION

It is not surprising that leaves sprayed with Bordeaux mixture should be cooler than unsprayed leaves. The film of spray gives the leaf a whitish cast which reflects the light rays, thus acting as a shade over the sprayed leaf. That the whitest Bordeaux reflects the most light is evidenced by the fact that leaves sprayed with 4–6–50 Bordeaux, and leaves sprayed with lime alone were cooler than leaves sprayed with 4–4–50. This, perhaps, explains the benefits sometimes derived from adding extra lime to Bordeaux mixture for potato spraying during July and August. It might also be said that the lamp-black spray shades the leaf, but, in this case, the light rays are absorbed and, as a result, the leaves are hotter than unsprayed leaves.

The fact that potato leaves sprayed with Bordeaux mixture are cooler than unsprayed leaves may partly explain the benefits derived from spraying in certain localities during years when diseases and insects are not factors. Although the lowering of the leaf temperatures as reported in the experiments is not large it might be considerably greater outdoors during the summer when the sun's rays are more intense. Even a reduction of 1.45° C. should have a beneficial effect in regions where the average temperature is above the optimum for potatoes. Bushnell (1) found that a rise in temperature from 20° C. to 23° C. reduced the weight of tubers

per plant from 20.9 grams to 5.0 grams. If it is true that the lowering of the leaf temperature is beneficial to the potato, in sections where the average temperature is above the optimum for the potato, it is also probably true that, in regions where the average temperature is near the optimum, no beneficial effects would be manifest. This may explain why no benefits result from spraying in the absence of disease in certain sections of Europe, as reported by Lutman (10), and in the Upper Peninsula of Michigan, as stated by Kotila and Coons (9).

Since sprayed leaves are cooler than unsprayed leaves, one would expect their transpiration rates would also be lower. Such, however, apparently is not the case, since Duggar and Cooley (5), Duggar and Bonns (6), Martin (11), and Shive and Martin (14) have shown that, in general, plants sprayed with Bordeaux have a higher transpiration rate than unsprayed plants. In the paper of Duggar and Bonns it is pointed out that this increase in transpiration occurs during the night interval and that during bright, sunshiny days the sprayed and unsprayed potato plants transpire about the same. In view of the fact that Clum (3) was unable to correlate small variations in leaf temperature with corresponding variations in transpiration, it is not surprising that the sprayed leaves, although cooler, do not have a corresponding lower transpiration rate.

SUMMARY

1. A study of the effect of copper sprays and dusts on the internal temperature of potato leaflets has been made.

2. Bordeaux mixture and copper-lime dust have a slight cooling effect on potato leaflets.

3. The whiter the coating of Bordeaux, the greater is the cooling effect. Leaves sprayed with 4–6–50 Bordeaux or with lime suspension are cooler than leaves sprayed with 4–4–50.

4. A dark colored spray increased the internal temperature of sprayed leaflets.

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THE VALUE OF PHYSIOLOGIC-FORM SURVEYS IN THE STUDY OF THE EPIDEMIOLOGY OF STEM RUST[1]

E. C. Stakman, M. N. Levine, and J. M. Wallace

In the spring of 1917, following the terrific stem-rust epidemic of the previous season in the hard-red-spring-wheat region of the United States and Canada, the Office of Cereal Crops and Diseases of the United States Department of Agriculture, in cooperation with several State experiment stations, began a comprehensive study of the epidemiology of cereal rusts, especially stem rust, *Puccinia graminis* Pers. One of the principal objects was to determine the sources of stem rust in the spring in the spring-wheat region of the upper Mississippi Valley, where the disease does the greatest damage.

It was considered that there were three possible sources of stem rust in the spring: 1, urediniospores which survived the winter on fall-sown grains and on grasses within the region; 2, aeciospores from barberry bushes; 3, wind-borne urediniospores from the South. From extensive experiments and observations it has been found that in the northern grain-growing States the uredinial stage of the rust does not persist from one crop season to the next except occasionally under unusually favorable conditions in limited areas.[2] For practical purposes this source of rust can be disregarded. As early as 1917 it became perfectly evident, on the other hand, that in the spring-wheat region much, if not most, of the rust in the spring came from barberry bushes (4, 7). The question remained whether the barberry was solely responsible for the development of epidemics or whether the uredinial stage could survive the winter in the southern United States and in the spring produce large numbers of urediniospores which might be blown northward in early summer and cause widespread infection in the spring wheat area.

As a result of overwintering studies, it has been shown that the uredinial stage of stem rust usually persists through the winter in the general district around San Antonio, Texas, but seldom so far north as Dallas, Texas (1). By the middle of May the uredinial stage of the rust usually is abundant

[1] Cooperative investigations between the Office of Cereal Crops and Diseases, United States Department of Agriculture, and the Minnesota Agricultural Experiment Station. Published with the approval of the Director as Paper No. 848 of the journal series of the Minnesota Agricultural Experiment Station.

[2] The general statements in this paper, unless otherwise specified, are based on personal observations and detailed experiments made in connection with the general rust-epidemiology study. Most of the results have not yet been published in detail, but they are available in summarized reports on file in the Office of Cereal Crops and Diseases and at the Minnesota Agricultural Experiment Station.

enough in the wheat-growing portions of Texas to furnish a large amount of potential inoculum for the grain fields of the North. Therefore attempts were made to ascertain whether these southern urediniospores could be blown northward early enough in the season and in sufficient quantity to contribute significantly to the development of epidemics.

Three principal methods were used in attempting to answer this question. Direct observations were made on the progressive development of stem rust from Texas northward in the spring and early summer; spore traps were exposed near the ground, from high buildings, and from airplanes and balloons, in order to obtain evidence regarding the number of spores in the air in regions where rust had not yet appeared (5); and physiologic-form surveys of *Puccinia graminis tritici* were made each year in order to find out whether the forms in the South were the same as those in the North. If the forms in the South were the same as those farther north, there would be circumstantial evidence that the rust might have been blown northward. On the other hand, if the forms in the South were distinctly different from those in the North, there would be presumptive evidence that the epidemics in the North could not have resulted from spores blown northward by the wind.

Final conclusions could not be drawn from the direct observations on the progressive development of rust from south to north. In some seasons rust in the North appeared to come almost solely from barberry bushes, whereas in other seasons there was evidence of migration of urediniospores from south to north. The migration apparently was gradual in some seasons and sudden in others. It seemed that in some seasons the urediniospores probably were disseminated relatively short distances from areas where rust was prevalent, and the northward movement appeared to be gradual. In other years the urediniospores seemed to be disseminated over a wide area in a short time. For example, in 1925, strong winds originating in Mexico, Texas, and Oklahoma blew northward almost continuously for about a week early in June. Within about a week after the first rains following the wind there was wide-spread and rather uniform infection of wheat in the spring-wheat area. This was strong circumstantial evidence that the south winds had disseminated urediniospores over a wide area from Texas to the Canadian boundary or beyond. But in some years *P. graminis avenae* seemed to have migrated from the South to the North, while *P. graminis tritici* did not; in other years the reverse was true. And even in years when both seemed to migrate, there was no evidence that the urediniospores of *P. glumarum* (Schm.) Eriks. and Henn. and of *P. coronata* Corda were blown northward, except for the latter in 1927.

It was decided, therefore, to intensify physiologic form surveys which had been made each year since 1917, because of their value in the problem

of breeding rust-resistant varieties and in predicting the probable efficacy of the barberry eradication campaign as a rust control measure.[3]

The results obtained soon explained why certain varieties of wheat sometimes were resistant in one locality and susceptible in another in the same year, or resistant in the same locality in some years and susceptible in others. In 1917 and 1918 H. D. Barker and the senior writer observed that several varieties of hard spring wheat, quite susceptible in the hard spring-wheat region, were highly resistant to the forms of *P. graminis tritici* which appeared to be most prevalent in the Southern States. For example, Marquis, C. I. 3641, Haynes Bluestem, C. I. 2874, and Glyndon, Minn. 163, remained free from stem rust when grown at Auburn, Ala., Bay Minette, Ala., and Baton Rouge, La., whereas certain other varieties, such as Alabama Bluestem, became heavily rusted when grown in neighboring plots. It was shown clearly by inoculation experiments in the greenhouse that this apparent immunity of the hard spring wheats was due merely to the absence of forms of *P. graminis tritici* which could infect them normally. For about two years the writers found no forms in the Gulf States which attacked the hard spring wheats normally. For example, in the spring of 1917 collections were made at Baton Rouge, La., Brundige, Ala., and Beaumont, Tex. All of them proved to be what was then known as *P. graminis tritici-compacti*, distinguished from ordinary *P. graminis tritici* primarily by its inability to infect hard spring wheats normally. Stakman and Hoerner (6, p. 148) made the following statement regarding this so-called form of rust, which in reality probably comprised several of the physiologic forms of *P. graminis tritici* subsequently described by Stakman and Levine (8) and now designated by numbers:

"The factors limiting the distribution of the rust and its possible importance in rust epiphytology are not known. It is possible that it is common only where soft wheats are grown, or where climatic factors may determine its geographic limits. If it proves to be wide-spread in the South it certainly renders less probable the hypothesis, held by many, of the seasonal spread of wheat stem-rust from south to north. It may also explain the fact that varieties of wheat show varying resistance in different regions. The whole question of the degree of constancy in the relations between the rust and its host plants is being investigated in connection with the rust epiphytology studies now being made by the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture."

[3] Physiologic form surveys have been made of *P. graminis avenae* also, but the results did not aid directly in the solution of the present problem, and therefore are not included in this paper. Similar surveys have been made since 1919 by the Dominion Rust Research Laboratory of Canada (2).

In 1918 it was found, however, that there were forms of *P. graminis tritici* in the South which could infect the hard spring wheats heavily, although it still seemed that those that could not so infect probably were more prevalent. Investigations therefore were made to ascertain whether the urediniospores of these forms were blown northward in the spring in sufficient numbers to cause epidemics in the spring-wheat region. The area in which the most intensive survey was made comprises Texas, Oklahoma, Kansas, Nebraska, Missouri, Iowa, Minnesota, South Dakota, and North Dakota.

The results obtained for several years were somewhat inconclusive, but those obtained in 1926, 1927, and 1928 seem significant and therefore are presented here in a preliminary way. A more detailed presentation of results will be made at a later time. The data are given in table 1 and figures 1 and 2.

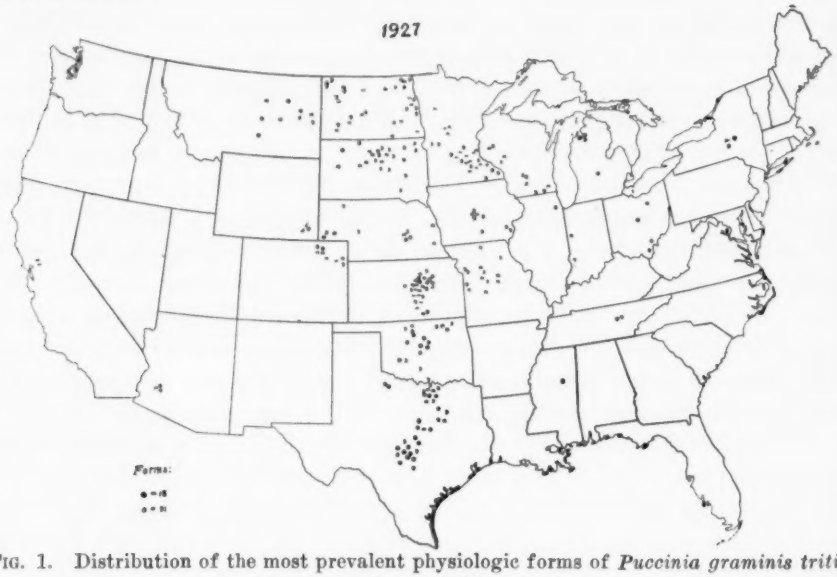

FIG. 1. Distribution of the most prevalent physiologic forms of *Puccinia graminis tritici* (forms 18 and 21) isolated in 1927.

It will be seen from table 1 and figure 2 that form 11 was fairly common in Texas in 1926, but it was not found in any of the spring-wheat States. Peltier and Thiel (3) state, however, that 13 of the collections which they made in Nebraska that year proved to be form 11. Possibly, therefore, form 11 migrated as far north as Nebraska but not so far as the spring-wheat area, although we may of course have missed it in our survey of the spring-wheat area. It also is possible that this form may have overwintered in the telial stage near barberry bushes in Nebraska and that urediniospores were not blown into the State from the South. This

TABLE 1.—*Distribution and prevalence of physiologic forms of Puccinia graminis tritici as indicated by the number of collections of each of several forms made in the Mississippi Valley in 1926 and 1927, and in Mexico and the Mississippi Valley in 1928*

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Distribution	Physiologic forms by number																					Total number of	
	1	2	3	11	17	18	19	21	23	28	29	32	33	34	36	38	39	49	50	51	52	56	Cultures	Forms
1926																								
Texas	—	—	—	6	1	1	1	2	—	—	—	—	—	—	2	—	4	—	—	—	—	—	17	7
Oklahoma	—	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	2
Kansas	—	—	—	—	—	1	—	1	—	—	—	—	—	1	2	—	—	—	—	—	—	—	5	4
Nebraska	—	—	—	—	—	2	—	2	—	—	—	—	—	—	4	—	—	—	—	—	—	—	8	3
Missouri	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1
Iowa	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	1
South Dakota	—	—	—	—	—	2	—	—	—	—	—	1	—	—	1	—	—	—	—	—	—	—	4	3
North Dakota	—	—	—	—	—	2	—	2	—	—	—	—	—	—	2	—	—	—	—	—	—	—	6	3
Minnesota	—	—	1	—	—	10	—	1	—	—	—	2	1	—	5	1	—	—	—	1	—	—	22	8
Total	—	—	2	6	2	21	1	8	—	—	—	3	1	1	16	1	4	—	—	1	—	—	67	13
1927																								
Texas	1	—	—	7	3	12	2	20	—	1	—	—	—	—	2	3	1	—	—	—	—	—	52	10
Oklahoma	2	—	—	—	1	8	—	10	—	—	—	1	—	—	2	1	2	1	—	—	—	—	28	9
Kansas	1	—	—	3	—	18	2	9	—	—	—	1	—	—	1	—	7	1	—	—	—	—	43	9
Nebraska	—	—	—	—	3	6	1	5	—	—	—	—	—	—	3	—	1	1	—	—	—	—	20	7
Missouri	—	—	—	1	1	7	—	6	—	—	—	—	—	—	—	—	2	1	—	—	—	—	18	6
Iowa	—	—	—	1	—	2	—	6	—	—	—	—	—	—	3	1	—	—	—	—	—	—	13	5
South Dakota	—	—	—	—	4	9	—	14	—	—	—	1	—	—	1	1	2	—	—	—	—	—	32	7
North Dakota	—	—	—	1	—	14	—	16	—	—	—	—	—	—	2	—	—	—	—	—	—	—	33	4
Minnesota	—	—	—	—	2	15	1	10	—	—	—	3	—	—	6	—	1	2	—	—	—	—	40	8
Total	4	—	—	13	14	91	6	96	—	1	—	6	—	—	20	6	16	6	—	—	—	—	279	12
1928																								
Mexico	—	—	—	1	5	—	—	2	3	—	—	1	—	—	1	39	2	5	1	—	1	—	61	11
Texas	—	—	—	2	—	—	1	7	—	—	1	2	—	—	5	20	—	4	—	—	—	—	42	8
Oklahoma	—	—	—	—	—	1	2	2	—	—	—	1	—	—	3	8	—	4	—	—	—	—	21	7
Kansas	1	—	—	5	—	3	2	9	—	—	—	—	—	—	10	2	4	3	—	—	—	—	39	9
Nebraska	—	—	—	—	1	—	—	8	—	—	—	4	—	—	8	4	3	1	—	—	—	—	29	7
Missouri	—	—	—	—	1	—	—	1	—	—	—	1	—	—	5	—	—	1	—	—	—	—	9	5
Iowa	1	—	—	—	2	1	—	4	—	—	1	—	—	—	8	1	4	1	—	—	1	2	26	11
South Dakota	—	—	—	—	—	1	1	9	—	—	—	—	—	1	16	2	1	1	—	—	—	—	32	8
North Dakota	1	—	—	—	2	—	—	3	—	—	—	1	—	—	8	1	—	1	—	—	—	—	17	7
Minnesota	2	1	—	—	1	1	1	15	—	—	1	1	—	—	18	1	—	5	—	—	1	—	48	12
Total	5	1	—	8	12	7	7	60	3	—	3	11	—	1	82	78	14	26	1	—	3	2	324	20

explanation seems plausible in view of the fact that Peltier and Thiel obtained some of their collections of form 11 near barberry bushes. It may be possible also that some of the collections identified by Peltier and Thiel

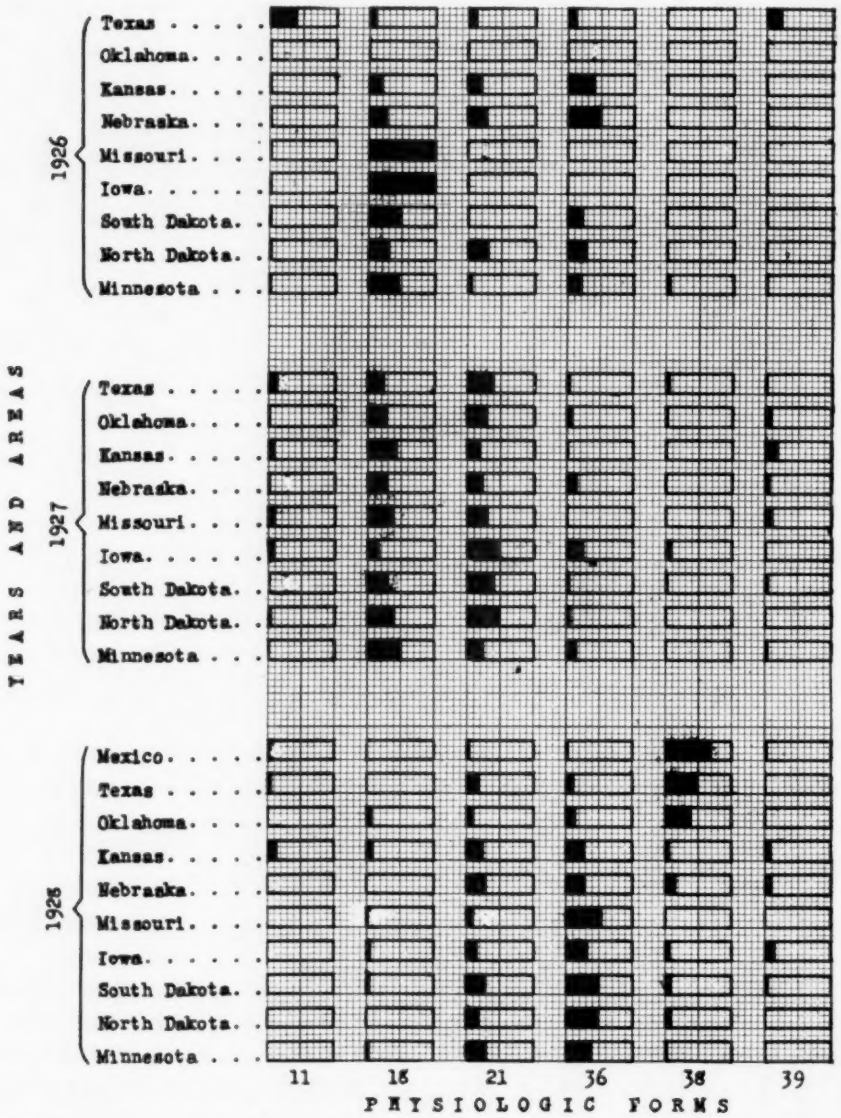

FIG. 2. Proportional distribution (per cent of total number of isolations in each case) of the most prevalent physiologic forms of *Puccinia graminis tritici* in the Mississippi Valley in the years 1926, 1927, and 1928.

as form 11 may have been form 32, as the two differ only in minor respects which are not always apparent. Form 11 produces a type 4 infection on the durum varieties used as differential hosts, whereas form 32 produces a type x infection, which may appear like type 4 unless environmental conditions are very favorable for the development of the type x infection. Unless several series of inoculations are made, therefore, form 32 might quite naturally be identified as form 11.

In any case, the most prevalent forms in the North were clearly 18 and 36, whereas those in Texas were 11 and 39. If these results can be relied upon, therefore—and the writers think they can—it is very unlikely that much rust was blown northward, because form 11 attacks the hard spring wheats and the durums heavily and there is no known reason why it should not have developed abundantly if it had made its way into the Northern States.

The results in 1927 were quite different from those in 1926. In 1927 a large number of collections were identified, and forms 18 and 21 clearly were the most outstandingly prevalent. As they appeared to be widely distributed from Texas northward, there is nothing in the results of the survey which would indicate that rust might not have been blown northward in sufficient quantity to account, at least in part, for the rust epidemic in the North.

The 1928 results are perhaps the most interesting of all. According to unpublished studies made by Wallace Butler, the uredinial stage of stem rust of wheat did not survive the winter in abundance even in the San Antonio district of Texas during the winter of 1927–28. Furthermore, the nature of the primary infection in Texas in the spring indicated that the rust had been blown into Texas from farther south. The most probable source, of course, would be Mexico. Collections were made in Mexico, therefore, as well as in the States from Texas northward. Of 61 Mexican collections, 39 proved to be form 38, and 3 proved to be form 23. Form 23 was not found at all in the North, but form 38 was quite abundant in Texas and Oklahoma. It also was found a few times farther north. Probably, therefore, this form of rust may have been blown from Mexico into Texas and Oklahoma and thence northward. But form 38 produces only very weak infection on the hard spring wheats, although it produces a type x infection on the durums. Had the only source of rust in the spring-wheat region, therefore, been that in the South, it seems very improbable that an epidemic could have developed even had weather conditions been exceptionally favorable. As a matter of actual fact there was no wide-spread epidemic of stem rust on wheat in the spring-wheat region in 1928. Whether this was because of the relatively small amount of effective inoculum which

came from the South or because of unfavorable weather conditions for rust development in the North, can not be stated with certainty. It seems significant, however, that in this year the form of rust which was by far the most abundant in the far South was relatively innocuous as far as the wheats in the spring-wheat region were concerned.

The results of the survey in 1928 suggest the possibility that physiologic-form surveys may be used in the future not only to explain certain facts in connection with the development of epidemics, as they already have done, but also to predict the probable occurrence of epidemics. When most of the barberries have been eradicated from the spring-wheat region, there probably will be only one important source of inoculum in the spring, namely, the urediniospores in Mexico and Texas.[4] The rust ordinarily develops fairly abundantly in Mexico during March and April, and by May it usually is abundant in Texas. It is entirely possible, then, that surveys could be made to determine which physiologic forms are present in the far South in time to be of some value in predicting the probability of epidemics in the North. Other factors besides the virulence of physiologic forms are important in governing the development of rust epidemics, but certainly if the forms of rust in the South in any given year are predominantly those which can not attack the wheats grown in the North, there would be little to fear even though the weather conditions were favorable for epidemics. On the other hand, if the forms of rust in the South were such as could attack the spring wheats normally, there would still be no way of predicting the likelihood of epidemics. It could merely be stated that an epidemic might occur if there were strong and long-prevalent south winds and if weather conditions were especially favorable for the development of rust in the North.

The results of the surveys thus far made show that the prevalence of physiologic forms in certain regions may vary greatly from year to year. In so far as these surveys bear on the question of the source of rust in the spring-wheat region, the following conclusions are justified. In some years the same physiologic forms seem to predominate throughout the wheat-growing region of the Mississippi basin from Texas to the Canadian border. This was true in 1927, and in some previous years. In 1926, however, the form of rust most prevalent in Texas did not make its appearance in the hard spring-wheat area. Therefore, it is unlikely that there could have been an important south-to-north migration of rust. In 1928 the rust may have been blown northward and probably was, but fortunately the forms then prevalent in the South could not infect the hard spring wheats.

[4] There is a possibility that rusted barberry bushes in the New England and other Eastern States may furnish inoculum also, but this possibility seems somewhat remote.

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A NEW SCLEROSPORA FROM FIJI

WILLIAM H. WESTON, JR.[1]

As our knowledge of the genus Sclerospora increases, the species which are found infecting wild grasses in less intensively cultivated parts of the world merit special attention as possible reservoirs of infection for related crops or as possible examples of endemism. It was, therefore, with special interest that the writer learned from Mr. D. S. North, Pathologist of the Colonial Sugar Refining Company, that in the Fiji Islands a leaf-splitting disease caused by a Sclerospora occurs on the common native "reed," *Erianthus maximus* var. *Seemanni* Hack. Through the kindness of Mr. North, material collected by H. F. Clarke on the Rarawai Estate of the C. S. R. Co. in Suva, Fiji, on June 23, 1924, was forwarded for examination. As the Sclerospora proved to be a new species quite distinct from the *S. sacchari* Miyake which occurs sporadically on sugar cane in Fiji a brief discussion of its peculiarities and its relationship is presented here.

EFFECT ON THE HOST

The Erianthus plants show the effects of the fungus by becoming generally dried, withered, and brown; while the leaves, as the tissue between their bundles is disorganized through the invasion of the parasite, shred out into shrivelled brownish fibers which tangle into irregular snarls. In general, this effect agrees closely with that of the oogonial phase of most Sclerosporas on their various hosts—for example, *S. sacchari* on sugar cane, *S. miscanthi* Miyake on Miscanthus, or *S. graminicola* (Sacc.) Schr. on Setaria (Chaetochloa).

As in these other cases, also, the resting spores develop in great quantities in the frayed-out leaves of the Erianthus and can be seen with a lens as distinct resinous specks in the disorganized tissue fragments along the tangled fibers. Of the once abundant mycelium which gave rise to these spores only a few shrivelled irregular fragments now remain here and there among the host cells.

No conidial phase of the fungus has yet been encountered.

CHARACTERISTICS OF THE FUNGUS

The resting spore, as is characteristic of the genus, comprises a single oospore enclosed in a much modified, darkened and thickened oogonial wall so closely adherent that the whole forms a single resistant body.

[1] The writer acknowledges with gratitude aid from the Milton Fund of Harvard University which has facilitated this and other comparative studies of the Sclerosporas.

The shape usually is approximately polyhedral, that of a sphere with several flattened faces (Fig. 1; B 1–3, C 1–5) bordered by projecting ridges (Fig. 1; B 6–8, 11, 12, C 3, 6–8) so that in profile it appears somewhat irregularly angular with wing- or fold-like protrusions, here and there (Fig. 1; B 6–8, 11, 12, C 3, 6–8). Occasionally the shape is more irregular, elongate pyriform (Fig. 1; B 4, C 7), or unequally rounded oblong (Fig. 1; B 12, C 6, 8). The developing oogonia are modified in shape and in surface configuration in conformity to adjacent spores and to contiguous host tissue. In some cases they occur in rows or groups closely

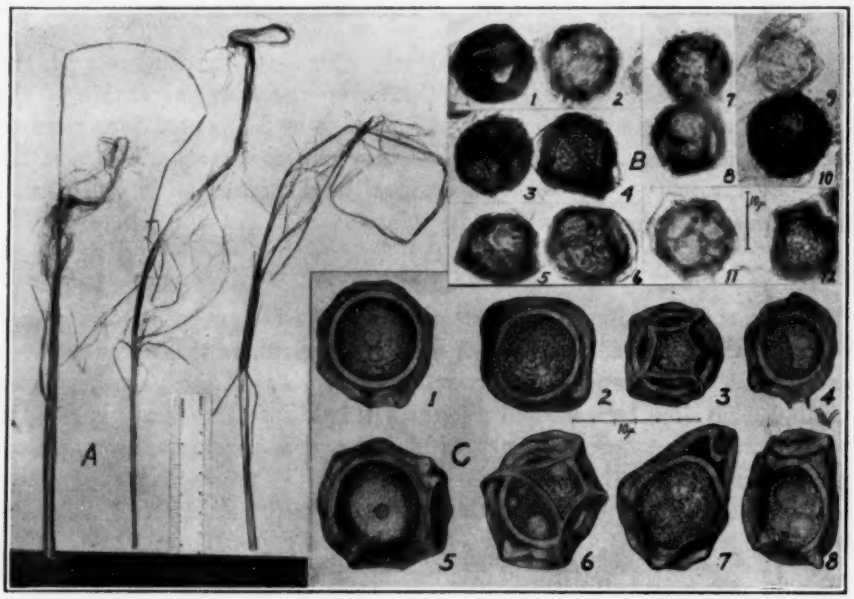

FIG. 1. A. Specimens of *Erianthus maximus* var. *Scemanni* infected by **Sclerospora northi** n. sp., from material collected by H. F. Clarke on the Rarawai Estate, Suva, Fiji, in June, 1924, showing the shredding of the leaves into snarls of vascular bundles from which the resting spores are scattered by every slight movement. Photographs, scale in inches and centimeters. B. 1–3, 7, 11. Resting spores showing the surface aspect with flattened polyhedral faces and irregular winged or ridged projections; 4–6, 8–10, 12, resting spores in optical section, showing the shape and size of the single enclosed oospore, and the characters of its wall and content; 9, a young spore in which the oogonial wall is not yet thickened, colored, and opaque, shows the interior especially clearly. Photomicrographs, scale in microns, magnification about 250 x. C. 1, 2, 4, 5. Resting spores, in optical section, showing the structure, thickness and profile of the oogonial wall, the shape and structure of the enclosed oospore and its proportions and position in relation to the enveloping wall; in 3, 6–8, combined surface and section views of cleared material show also the typical superficial configuration. Camera-lucida drawings, scale in microns, magnification about 375 x.

pressed together and with faces of contact that more or less fit each other (Fig. 1; B 7–8, C 5–6). As seen in optical section the general outline of the enveloping oogonial wall may conform approximately to that of the spherical oospore within (Fig. 1; C 1, 2, 4, 5), but the wall itself is not everywhere in intimate contact with the enclosed oospore, but arches, bulges, and folds outward into irregular, ridged prominences, leaving corresponding spaces between its inner surface and the oospore wall (Fig. 1; C 6–8). Moreover, in addition to being outfolded, the wall is rendered still more irregular through being locally decidedly thickened, at times in noticeable layers, and the surface frequently shows indefinite scabrous patches or angular or ridged protrusions.

The thickness of the oogonial wall varies, but usually is from 3 to 5 μ, less frequently in occasional thickened areas reaching 10 μ.

In color, the wall of mature spores is most commonly a dark amber, the Amber Brown, 13 k of Ridgway's "Color Standards," though ranging from the lighter Raw Sienna, 17 i, to the dark Argus Brown, 13 m. In

TABLE 1.—*Measurements of the outer dimensions of 200 resting spores of* **Sclerospora northi** *n. sp. and diameters of the enclosed oospores arranged in size classes to show frequencies*

Outer dimensions of resting spores			Diameters of oospores	
Classes	Number of resting spores in 200		Classes	Number of oospores in 200
μ	Length	Width	μ	
39 to 40.9		1		
41 to 42.9		1		
43 to 44.9	1	4		
45 to 46.7	2	9	35 to 36.9	7
47 to 48.9	4	25	37 to 38.9	16
49 to 50.9	11	23	39 to 40.9	35
51 to 52.9	2	20	41 to 42.9	43
53 to 54.9	21	46	43 to 44.9	41
55 to 56.9	19	32	45 to 46.9	30
57 to 58.9	38	20	47 to 48.9	19
59 to 60.9	28	9	49 to 50.9	8
61 to 62.9	27	7	51 to 52.9	1
63 to 64.9	17	2		
65 to 66.9	11	1		
67 to 68.9	10			
69 to 70.9	4			
71 to 72.9	1			
73 to 74.9	1			
75 to 76.9	0			
77 to 78.9	1			

darker individuals the wall can be seen through only with difficulty, even with intense illumination; in immature ones, with their pallid golden to amber color, details of the structure can be perceived with ease.

Remains of the oogonial stalk persist but rarely (Fig. 1; C 4), and only seldom are to be seen scars or impressions which might be interpreted as points where an antheridium had been attached; but, as fertilization apparently occurs very early, the thickening of the wall usually obliterates them.

The outside size of the resting spores varies greatly, ranging from as little as 40 to as much as 77 μ, with the greatest dimension, "length," falling most commonly between 57 and 60.9 μ, the dimension at right angles, "width" most frequently from 51 to 56.9 μ.

The oospore, as seen in optical section usually is central in position, of relatively large size in relation to the enclosing oogonium, and spherical in form, only rarely, in irregular resting spores being somewhat flattened or elongated, apparently restricted or constrained by the confined space in which the body has developed (Fig. 1; C 7, 8). In size the oospores are rather uniform, for, although a few may run as small as 35 μ or as large as 52 μ in diameter, the bulk of the individuals measured (*i.e.*, 154 out of 200), fall between 39 and 46.9 μ, with the size mode falling in the class (containing 84 spores) between 41 and 44.9 μ. The oospore wall is tough, dense, smooth, homogeneous or perhaps indistinctly lamellate, either hyaline or faintly tinged with pale amber color, uniformly thick, quite consistently measuring from 2 to 4.5 μ. The content of the oospore is hyaline or pallid grayish with a finely granular protoplasmic matrix in which lie denser aggregations of granules and with a clearer or vacuolate area, usually in the center, in which, in some cases, one or more refractive, perhaps golden tinged oil globules may be seen (Fig. 1; B 4, 6, C 1, 5–8).

The germination of the oospores has not been observed.

IDENTITY OF THE FUNGUS

Of the known Sclerosporas the *S. sacchari* and the *S. miscanthi* of T. Miyake in their oogonial stage show sufficient general similarity to this Fijian species to invite comparison.

S. sacchari, however, is distinguished by the fact that the resting spores are more nearly spherical, less polyhedral in shape, the oogonial wall usually much thinner, more closely conforming to the oospore within, the surface more distinctly scabrous or irregularly roughened, with ridges or winged projections, if present, usually less prominent and occupying a relatively small area, its material more transparent and of paler golden or lighter amber color. The oospores, moreover, are distinctly larger, most frequently between 49 and 54.9 μ in diameter, and with a much thicker wall, 5 to 8 μ. In like manner, though less pronounced, may be distinguished the

oogonial Sclerospora occurring on *Saccharum spontaneum* L. in the Philippines, a species very similar to *S. sacchari* but perhaps separate from it.

S. miscanthi of all the Sclerosporas, is the one the Fijian species resembles most closely, the general appearance of the resting spores, their shape, surface characters and color; and the proportion and relation of the oospore to the enveloping wall, being vary similar. Yet *S. miscanthi* seems consistently in all the material examined to have distinctly more rounded, less polyhedrally angled resting spores, and oospores which not only occupy a relatively greater space and more nearly fill the oogonial cavity but are distinguishably larger, the most of those encountered in the hundreds measured being between 47 and 48.9 μ in diameter.

With species that show such general similarity it is obviously desirable that cross-inoculation studies be made to determine whether this Fijian species may be transferred from Erianthus to sugar cane, to *Saccharum spontaneum*, to *Miscanthus japonicus* (Thunb.) Ander., and other members of the Andropogoneae, and if so whether it will retain its specific characteristics when developing on these hosts.

As far as can be determined at present, however, it seems justifiable to establish this as a distinct species. It is, therefore, described as follows:

Sclerospora northi n. sp.

Oogonium (outer portion of the resting spore) usually rounded polyhedral in shape, with several flattened faces bordered by ridges, occasionally more irregular, elongate pyriform, or unequally rounded oblong; in size ranging from 40 to 70 μ, greatest dimension, "length," most frequently between 57 and 60.9 μ, dimension at right angles, "width," most frequently 51 to 56.9 μ. Oogonial wall arched and folded out into irregular, ridged prominences leaving corresponding spaces over the oospore wall, in thickness usually 3 to 5 μ, occasionally as much as 10 μ. Wall most commonly Amber Brown, 13 k, of Ridgway, but ranging from Raw Sienna, 17 i, to Argus Brown, 13 m. Remains of oogonial stalk or of attached antheridium rare.

Oospores regularly spherical, most frequently from 39 to 46.9 μ in diameter, the mode between 41 and 44.9 μ, the extremes ranging from 35 to 52 μ; wall hyaline to pale amber, dense, smooth, homogeneous to indistinctly lamellate, 2 to 4.5 μ in thickness; content finely granular with denser aggregations and with clear usually central area with occasionally one or more oil globules.

Germination not yet observed.

Occurring on *Erianthus maximus* var. *Seemanni*, in the Fiji Islands; material collected by H. F. Clarke on the Rarawai Estate, Suva, Fiji, June 23, 1924.

Specimens deposited in the Farlow Herbarium, Harvard University.

DISCUSSION

In connection with this new Fijian Sclerospora certain points are worthy of note.

Its occurrence on an Erianthus is of interest, for the genus, on which hitherto no species of Sclerospora has ever been reported, must now be added to the long list of grasses that are known to be attacked by these mildews. It does not seem surprising, however, that this should be so, for, in the tribe Andropogoneae, the two genera, Miscanthus and Saccharum, both closely related to Erianthus, have been found to harbor two, possibly three, species of Sclerospora under natural conditions and to be susceptible to inoculation with two more.

Of the grasses of the Fiji Islands, Seemann, in his "Flora Vitiensis," of 1865, listed fifteen species, among them *Miscanthus japonicus* (*Eulalia japonica* Trin.), and sugar cane *Saccharum officinarum* L., of which he says "a purple variety attaining a height of 16 feet and of corresponding thickness is cultivated to some extent". His statement that this cane "grows, as it were, wild in various parts of the group," refers more probably to *Saccharum spontaneum* which, according to D. S. North, occurs rather commonly there. On Seemann's "*Saccharum floridum,*" No. 691, there was established by Hackel, *Erianthus maximus* var. *Seemanni,* the variety reported only from Fiji, the species itself known from Tahiti and the Society Islands as well. However, the most recent discussion of the grasses of the Fijis by Summerhays and Hubbard enumerates 59 species, but does not include the Erianthus nor any reference to Seemann's specimen on which this variety was established.

Of the Gramineae just noted, *Miscanthus japonicus* is known to harbor *Sclerospora miscanthi* in Formosa and the Philippines, sugar cane is infected by *S. sacchari* in Formosa, the Philippines, and Australia, while on *Saccharum spontaneum* the writer found a Sclerospora related to *S. sacchari* occurring commonly throughout the Philippine Islands.

In the Fiji Islands the following Sclerospora have been found by Mr. D. S. North:—The Sclerospora on the native "reed," *Erianthus maximus* var. *Seemanni,* herewith described as *S. northi;* a Sclerospora causing a leaf-splitting disease of "Veico", the wild cane (probably *Saccharum spontaneum*) which, although not yet secured for study, is perhaps the same as that which occurs in the Philippines on this host, and a Sclerospora occurring in sporadic cases on cultivated sugar cane, formerly assumed to be *S. sacchari,* but now thought by North to be distinct, as it does not cause elongation of the shoots, rarely shows the downy conidial phase, and produces oospores more readily, accompanied by more leaf splitting.

With fungi of such potentialities occurring among such closely related hosts in so small and isolated an area as the Fiji group, it seems especially

desirable that cross-inoculation experiments should be made to determine whether the species on sugar cane and *Saccharum spontaneum* are identical or are distinct; whether all these species are restricted to their respective hosts or may transfer from one to another under natural conditions or under special circumstances; and whether centers of infection on the various hosts may serve as foci from which the several fungi may pass to some cultivated crops in later agricultural developments.

As *Sclerospora northi,* so fas as known, is limited to this particular variety of Erianthus in this small group of islands, one might assume that it is endemic, but such an assumption is unjustified in the present lack of information as to the occurrence of downy mildews on *Erianthus maximus* Brongn., itself, in Tahiti and the Society Islands.

The disease caused by *S. northi,* having the same general leaf-splitting symptoms as those of the better-known malady of sugar cane, when first recognized, naturally roused the suspicion that the two might be the same and that this native "reed" growing around the plantations might serve as a reservoir of infection, greatly complicating the problem of combating cane losses. The certainty that this is a distinct species frees it from suspicion and removes it from consideration in the control of sugar-cane mildew.

As far as known, therefore, this new species is not, at present, of any economic importance as it is not seriously destructive to its wild host, nor is it passing therefrom to more valuable cultivated crops.

SUMMARY

This paper discusses a Sclerospora causing a leaf-splitting disease of *Erianthus maximus* var. *Seemanni* in the Fiji Islands.

In its resting-spore stage, the only reproductive phase yet encountered, the fungus seems distinct from *S. sacchari* and *S. miscanthi,* the two species most similar to it. It is, therefore, described as *S. northi* n. sp. and its characteristics of structure, size, and surface configuration are discussed.

The present status of this and related Sclerosporas in the Fiji Islands in regard to questions of distribution, relation to hosts, and control of disease is noted.

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PLANT–DISEASE NOTES FROM THE CENTRAL ANDES II

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In a recent paper by Chardon and Toro (*1*) an account was presented of the plant diseases encountered by Chardon during his visit to the State of Antioquia in Colombia. Thirteen diseases were reported, of which ten were hitherto not known to occur here. The present paper is based on the observations of the writer during the past year. The majority of the diseases cited are new to Colombia, while additional data are given regarding the etiology and ecological relationship of the coffee-root disease and the symptomatology of avocado leaf spot and data on other diseases.

An account of these follows:

Coffee-root disease. Studies of this disease under field conditions seem to indicate the presence of two different diseases or, at least, two different sets of symptoms of the same disease, under the vernacular name of "llaga" (canker).

The first of these is a dry rot of the upper surface roots and of the base of the trunk. No visible symptoms of the disease appear until it is well advanced, when the leaves turn yellow and fall. In some cases, a few leaves remain attached to the tree, even though they have lost their green color. If the tree is bearing fruits, these remain on the branches but do not mature normally. Examinations of diseased parts disclosed mycelial strands of a fungus resembling those described by Fawcett (*2*) associated with the black type of coffee-root disease in Porto Rico. This disease, however, is not very prevalent. It was observed only in few plantations and, generally, weakened trees are the only ones to die. Strong, well-cared-for trees do not so easily succumb to attacks of the causual organism. The writer saw trees that, according to the manager of the plantation, had been infected for two years without showing any external symptoms of the disease.

In addition to coffee, several weed and shade trees are also attacked. Strands of mycelium similar to those observed on coffee were found on the stumps of old, dead Inga trees. In one case abundant perithecia of an unidentified species of Rosellinia were found. We know of no connection between this ascigerous stage and the mycelial strands found on coffee and other plants, but other investigators assert that such connection exists.

Weather conditions seem to have very little effect on the prevalence of the disease. Since there is always considerable moisture in the soil, due to the fact that coffee is grown under shade, the disease appears in wet as well as in dry weather. Too much wet weather, however, seems to check the spread of the fungus.

The other form of "llaga" (canker) is more interesting, although rarer; while in the former case the base of the trunk turns black, with part of the

bark disintegrating, thus exposing the wood, in the latter, the bark often remains attached and acquires a greenish color. Sometimes it turns soft and gives off a pungent odor. In the majority of the specimens of this second "llaga," a Fusarium, intermixed with bacteria, was present. In some specimens, in addition to the Fusarium and bacteria, the mycelial strands of the first form were also observed. The bark commonly, though not always, turns black or the wood becomes exposed.

In a few cases perithecia of Nectria were found on the stumps of coffee roots and stems which had died from this second form of "llaga". This Nectria is identical with Chardon's specimen (Fung. of Colombia, No. 101), from Fredonia, Antioquia, which was studied at the New York Botanical Garden by the author. It agrees with the description of *Nectria coccinea* (Pers.) Fr. var. *tropica* Wr. as described by Wollenweber (8), from coffee material collected in Brazil. Chardon's specimen, although agreeing with the description given by Wollenweber, is very distinct from *Nectria coccinea*, as shown by the specimens, from different localities, deposited in the herbarium of the New York Botanical Garden. The "contextu radiatifibrato peritheciorum" character, so peculiar of *N. coccinea* var. *tropica*, is entirely wanting on the specimens of *N. coccinea* examined. Also the spores in the variety are smaller than those shown by the species. In view of these distinctions we propose that the variety be considered a distinct species and rename it **Nectria tropica** (Wr.) comb. nov.

It remains to be proved whether there is any connection between the Fusarium associated with the second form of "llaga" and this Nectria, and whether this Fusarium is a primary pathogene or only invades wounds received by the trees during cultural operations. An investigation of these relationships has been initiated.

Cercospora spot of coffee. This disease was observed for the first time this year. In a great majority of the grains classed as second-grade, due to adherence of the parchment, lesions and spores of the Cercospora type were found. In the berries the fungus forms black blotches which may cover as much as half of the surface. These blotches occur more frequently on the upper side where the sun causes early ripening. In the leaves the spots are circular, at first brown, later becoming almost white in the center with a definite brown margin. When dry, the central part does not break away as happens in the case of the leaf spot caused by the fungus *Omphalia flavida* Maubl. & Rangel. The cause of this tronble is a fungus first described from Jamaica under the name of *Cercospora coffeicola* Berk. & Cke.

Field observations have shown that the infective capacity of the causal organism depends on conditions affecting the trees. The disease is more prevalent in places where the shade is insufficient, where cultivation has

been neglected, and where, in general, a weakened condition of the tree prevails.

Top rot of sugar cane. A serious outbreak of this disease was reported during the month of October on a farm at Bello, near Medellin. Other sporadic cases have been reported from Sonsón and other places. An examination of the field in Bello showed a loss of at least 60 per cent of the crop. At that time the weather was very rainy and the soil was full of stagnant water. At first, therefore, the trouble was thought due to excessive soil water. A closer examination of the stools showed that the plants were suffering from a disease similar to that described by Matz (*3*) as the dry top rot of sugar cane in Porto Rico. There was a wilting and drying of the central leaves, a stunting of the cane stalks, and a dry rot of the uppermost joints. The length of the internodes of some of the stalks was so reduced that there was an unusual number of nodes, while root development was greatly increased. Microscopical examination of the stalks showed the vascular bundles all clogged with orange red, spherical masses. This condition was observed in plants growing on the hilly parts of the plantation. Those growing in the valley showed the characteristic yellowing resulting from excessive moisture.

Since top rot may be caused by several organisms, the writer is not certain that the Antioquian disease is the same as that described by Matz from Porto Rico. The fact that a heavy infestation of borers is always present in all cane plantations in Antioquia and that several different fungi may cause the spherical, orange-red masses in the vascular bundles and other tissues of the cane stalk, makes it necessary that a more careful study of the disease be made before giving any definite conclusions as to its exact causation.

It is impossible to consider at the present time the matter of varietal resistance to the disease, since the four varieties planted here are the most susceptible to Matz's disease. These are the Otaheiti, Rayada, Christalina, and Cavangerie, the last one being the most resistant. Since cane production in Antioquia is dependent on these varieties—for lack of others to take their places—if the disease continues to check production and dissemination, it will be a matter of years before other cane varieties can be established and methods of control be developed.

Red rot of sugar cane. Several complaints came to the writer that the yields of "panela" (loaf-sugar) were considerably decreasing on account of a stunting and drying of the canes and the consequent small yields. Visits to some of the plantations showed that this disease was due to a number of causes, chief among which were defective cultural methods, enabling the facultative parasites to attack the already weakened plants. Examination of the roots of several of these sick plants showed a reddish

coloration accompanied by death of the great majority of them. A microscopic examination showed the presence of macroconidia of a *Thielaviopsis*, very probably *Th. paradoxa* (Des.) v. Hohn., the cause of the pineapple disease of sugar cane in other cane-producing countries.

This fungus is primarily a cutting-inhabiting organism and is the cause of much poor germination in cane fields. However, several cases are known in which the fungus has attacked standing canes, especially in wet weather. In cutting, the fungus enters through the wounds received during planting operations, but in standing cane it is difficult to ascertain how the organism enters the plant, unless it be through the borer holes or through wounds received by the plant from the attacks of other organisms or during cultural operations. In view of our knowledge of cane diseases in Antioquia, we are not in a position to express definite conclusions as to the exact cause of this red rot. The fact that root troubles in sugar cane may result from a number of causes and that, even though fungi are found associated with this trouble, which, under proper management of the plantation, are unable to cause any great losses, makes it the more difficult to ascertain the exact nature of the diseased conditions.

Avocado tar spot. This is a leaf spot caused by the fungus *Phyllachora gratissima* Rehm. In Antioquia it is the worst malady of the avocado, *Persea persea* (L.) Cock. It causes considerable falling of the leaves, in some cases killing the trees because of their inability to produce new folliage.

The fungus first makes its appearance on the young leaves at any time before they reach maturity, in many cases killing the young leaves before these have attained their full size. This results in reduced vigor and subsequent attack at the tops by secondary fungi. Black, conspicuous, shining spots appear on the upper side of the leaves with corresponding yellowish spots on the under side. The stromata are arranged in circles, sometimes separated by brownish zones, and so abundant as to cause the leaves to fall prematurely. In most cases the leaves turn brown before falling.

Leaf spot of cauliflower. During a visit to the Granizales section, the place where most of the vegetables consumed in Medellin are produced, several vegetable diseases were encountered. The most conspicuous of these was the black leaf spot of cauliflowers. The whole head was in many instances covered with a brownish black crust formed by the conidiophores and spores of the fungus *Alternaria brassicae* (Berk.) Sacc. Weather conditions seemed to favor the development of this organism, since plants which were exposed to the sun at all times, and consequently drier, were less affected by the disease.

Ring spot of cabbage. Concentric, circular, light brown spots, surrounded by greenish borders were observed on cabbage leaves. Closer examination of the spots showed them to be closely studded with perithecia of the fungus *Myosphaerella brassicicola* (Duby) Lindau.

Cercospora leaf spot of lettuce. A severe attack of leaf spot on lettuce was encountered. The organism found associated with it agrees with *Cercospora lactucae* Stevenson described from similar material in Porto Rico (*5*). Welles (*7*) also described another Cercospora on lettuce and named it *C. lactucae* Welles. Both of the descriptions agree with that of *C. longispora* Cug. as given by Traverso (*6*). The name *C. longispora* was previously occupied and Saccardo (*4*, p. 607) changed the name to *C. longissima* (Cug.) Sacc. P. Hennings described another Cercospora on lettuce, which he named *C. lactucae* P. Henn. before Stevenson and Welles described their species. *C. lactucae* P. Henn. is very distinct from *C. longissima*. Since *C. longissima* is an older name, the fungus from Porto Rico, Philippines, and Colombia should be referred to this name.

Leaf mold of tomato. Garden tomatoes were found heavily infested with the fungus *Cladosporium fulvum* Cke. The disease is more prevalent during rainy weather.

Bean anthracnose. All varieties of beans are attacked by the anthracnose fungus *Colletotrichum lindemuthianum* (Sacc. & Magn.) Brio. & Cav. The lesions on the pods differ according to the variety attacked and the prevailing climatic conditions.

Wither tip of mango. An investigation of the premature falling of flowers and of young fruits of mango (*Mangifera indica* L.) showed that a fungus *Colletotrichum gloeosporioides* Penz. was always associated with the trouble. Besides producing these symptoms, the fungus also causes a wither tip of the young branches, so that considerable damage is done to the tree accompanied by the very scarce production of fruits.

Bunchy top of carnation. Associated with this trouble was found the fungus *Heterosporium echinulatum* (Berk.) Cke. Young leaves were covered with a blackish crust formed by the conidiophores and spores of the fungus, while older leaves were dead and the plants acquired a broom-like appearance, due to abnormal growth of lateral branchlets.

Banana anthracnose. Heavy infections of fruit rots were observed on ripe market bananas. Abundant acervuli and spores of the fungus *Gloesporium musarum* Cke. & Massee were found on the lesions. It seems that this fungus is capable of causing the trouble on ripe fruits that have been injured.

Minor diseases. Among the other diseases occasionally observed were: blue mold of oranges caused by *Penicillium digitatum* (Pers.) Sacc.; leaf spot of pepper caused by *Cercospora capsici* H. & W.; flower-head blotch of the grass *Sporobolus berteroanus* (Trin.) H. & C. caused by *Helminthosporium ravenelli* Curt.; black leaf spot of *Carica papaya* L. caused by *Asperisporium caricae* (Speg.) Maub. This fungus has been reported under a variety of names such as *Cercospora caricae* Speg. and *Puccinio*p-

sis caricae Earle. A heavy infection of Inga leaves by the alga *Cephaleurus virescens* Kunze was observed during dry weather.

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ATTENUATION OF CURLY-TOP VIRUS BY RESISTANT SUGAR BEETS WHICH ARE SYMPTOMLESS CARRIERS

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INTRODUCTION

Since the previous work was reported on attenuation of curly-top virus by resistant sugar beets showing mild to moderately severe symptoms (3), other beets have been found in the field which attenuate the virus without showing any visible manifestation of its presence.

The first work on the attenuation of the virus of sugar-beet curly top was reported by Carsner and Stahl (4). They found *Chenopodium murale* L. to have a marked effect on the virus. Carsner (2) found that *Rumex crispus* L. and *Suaeda moquini* Greene also attenuated the virus when it was passed through them. Carsner and Stahl (4) failed to detect any well-defined symptoms of curly top on *C. murale*. In the writer's work, these observations are confirmed; numerous experiments indicate that under certain conditions *C. murale* not only attenuates the curly-top virus, but also acts as a symptomless carrier.

Several examples have been reported in which plants of one genus act as symptomless carriers of a virus affecting plants of other genera. Johnson (6) reports apparently healthy potatoes harboring a virus which causes a disease of tobacco. Elmer (5) and Nishimura (8) record cases in which *Physalis alkekengi* L. also harbored a virus of tobacco without apparent symptoms. Atanasoff (1) found that some varieties of potatoes may carry the virus of stipple streak without any visible effect, but that when the virus is transferred to potatoes of a different variety conspicuous symptoms may become visible. Except for the plants reported by Carsner and Stahl (4) and Carsner (2), no attenuation of the viruses harbored by these various hosts has been recorded.

While continuing some investigations on the attenuation of the curly-top virus by resistant sugar beets (3), some individual plants were discovered in the field, among the most resistant strains of sugar beets, which were apparently healthy, having no visible curly-top symptoms whatever. Each beet in the field had been inoculated by caging two viruliferous leaf hoppers (*Eutettix tenellus* Baker) on it for 24 hours while it was very young, and the plants had also been exposed to a fairly heavy natural infestation of leaf-hoppers. These inoculations might be expected to produce severe curly-top infection in plants of ordinary susceptibility and thus cause the apparently healthy individuals to attract attention. In this same

[1] The investigations herein reported were conducted under the supervision of Dr. Eubanks Carsner.

field, in all the other strains which are susceptible, or at least less resistant than the strains in which the seemingly healthy individuals occurred, all plants exhibited symptoms of curly top. It is evident from these facts that the symptomless plants had been well inoculated.

The facts discovered by other workers, as previously noted, regarding the harboring of viruses without apparent evidence of their presence (1, 6, 7, 8) suggested that a similar situation might exist in the case of the apparently healthy resistant beets. The fact also was kept in mind that they might have resisted infection entirely. Earlier research (3) indicated that when resistant beets act as carriers of the curly-top virus they may produce an attenuating effect. The severity of the symptoms on the plants inoculated corresponds rather closely to the degree of severity exhibited by the resistant beets from which the virus was taken. The possibility was therefore suggested that in case the virus was harbored by the symptom-free resistant beets here under consideration it might be extremely attenuated.

EXPERIMENTAL TESTS

Experiments were conducted to determine whether or not the virus was present in these apparently healthy sugar beets and, if present, the degree to which it was attenuated. Some of the youngest inner leaves were taken from these symptom-free sugar beets, and nonviruliferous leaf hoppers were caged on these detached leaves for 2 to 3 days. These insects were then caged in lots of 10 on healthy, young, four-leaf susceptible beets. For controls, similar plants were inoculated with leaf hoppers which had fed on severely diseased leaves of a susceptible beet. All inoculated plants were kept under conditions favorable for the development of curly top. In 10 to 37 days all the plants inoculated with virus from these symptomless carriers became diseased, but they were not so severely affected as the controls. The average incubation period of the control plants was shorter than that of the others, all controls showing the first symptoms in less than 10 days. There was a noticeable variation in the degree of attenuation among the different individuals in these strains of symptom-free beets. For example, in one case the virus produced moderately severe symptoms in 10 days on susceptible beets, although these symptoms were not so severe as those on the controls. In contrast to this the virus from another strain of resistant carriers produced mild and very inconspicuous symptoms of curly top in 37 days. Furthermore, about five weeks later, the faint symptoms on the mildly affected beet had disappeared entirely. It apparently had completely recovered. Repeated tests yielded similar results. However, in most cases the virus from the symptom-free beets produced symptoms almost as severe as the virus from the beets showing mild to moderately severe effects (3).

While, in breeding sugar beets for resistance to the curly-top virus, the appearance of symptomless carriers has never been such a confusing factor as has been the case with this phenomenon in certain other virus diseases, it seems quite possible that one not familiar with the fact of their occurrence might be led to consider such apparently healthy beets to be immune from the disease.

Aside from any practical application which the results of these experiments may have, they demonstrate the interesting fact that under certain conditions some resistant sugar beets acting as symptomless carriers may harbor the curly-top virus in a state almost as virulent as do those resistant beets which exhibit symptoms ranging from mild to moderately severe.

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